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Vol. 31 No. 2

Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

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APRIL, 1952

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Published for THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
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Vol. 31, No. 2, April, 1952. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription price: United States, its Possessions, Pan-American Countries, $9.00 per volume; Canada and other foreign countries, $9.50. Single number when available, $1.25. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the Act of March 3, 1879. Printed in the U. S. A.

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EDITOR'S VIEWPOINT

Nelsen and Kumpula (p. 158) describe a panographic radiographic technique and an apparatus for the technique. The importance of radiography in oral diagnosis is sufficient stimulus to search for an easier, less expensive, and time-saving method of examining the calcified oral structures. While the panographic method probably will never replace conventional methods for intraoral radiographs used for diagnosis, it may well bear the same relationship to the contemporary technique that the 35 mm. chest film bears to the large chest plate, i.e., in large group surveys panographic radiography may rapidly reveal those individuals who should be examined by conventional intraoral radiographs.

Fleming (p. 166) successfully transplanted tooth germs into the anterior chambers of animals of the same and different species. His results show the ability of the tooth germ to form in intraoral sites and that the ameloblasts and odontoblasts, once differentiated, have the ability to form enamel or dentin independent of each other. The resemblance of Fleming's illustrated sections to various intraoral anomalies, sometimes called neoplasms, and to mixed odontogenic tumors is striking.

The possible role of phosphatase in releasing phosphates has been discussed in relationship to dental caries. On the basis of a limited study it was suggested that bacteria made relatively little contribution to the acid phosphatase content of saliva but a more complete study by Fitzgerald (p. 189) indicates that certain oral bacteria may be rich sources of this enzyme. The agreement between salivary lactobacillus counts, Snyder test results, and the phosphatase content of saliva, lead Fitzgerald to suggest the use of this latter test as a faster and simpler index for caries susceptibility.

Massler, Ludwick, and Schour (p. 195) were unable to find any correlation, positive or negative, between gingivitis and caries experience. It should be pointed out that severity of gingivitis, as used by these authors, refers to the quantity of tissue involved rather than to the mildness or severity of attack by the disease process in a given area. We usually consider a severe stomatitis or gingivitis one which involves depth of tissue in a marked disease process rather than one which superficially involves most of the oral tissues or gingivae in a mild manner. To this extent PMA fails to record the true severity of disease.

Neumann, Lefkowitz, and DiSalvo (p. 200) conducted an interesting study of a group of 102 children who were required to chew sugar cane daily. This group showed approximately one-half the number of new cavities as were found in two control groups after 18 months. The authors are inclined to credit the decreased caries activity to physiologic changes in the teeth, a theory advanced by Neumann, and by Neumann and DiSalvo, in earlier publications, although the authors admit that the detergent action of the cane, or other factors, may be effective. The chewing of fibrous materials at the end of each meal has been suggested from time to time to reduce caries activity but has never been popularized. The authors point out that other methods of caries control, which are equally effective, are adopted more readily.

The problem of electric currents in the oral cavity has long been the subject of debate. A thorough investigation of the problem, from the viewpoint of physics, is reported by Schriever and Diamond (p. 205). They demonstrate the existence of such currents between different fillings and around individual fillings. They consider the "bone fluid," i.e., the electrolytes of the teeth, bone, and soft tissue, as important as the saliva. They suggest that pathologic lesions may be caused by electric currents through and around fillings but do not demonstrate such lesions. It is proposed that individual sensitivity of the patient to metallic ions may be more important than the magnitude of the current. The effect of "insulating" cements beween the metallic fillings and the "bone fluid" is not discussed nor is the

possible effect of dentin sclerosis. The establishment of the existence of the electromotive forces is significant and the techniques and apparatus described are worthy of application in studies of mouths with pathologic lesions presumably caused by electric currents about metallic fillings.

Dalton (p. 230) "interfered with the continuity of the rat maxilla" to investigate the healing process. This is the equivalent of fracturing the zygoma and firmly fixing the fracture. This direct approach demonstrated that the maxilla heals by bony union, although many had claimed that there is only fibrous union in that bone. Even in the presence of osteomyelitis, which occurred in some animals, there was bony union. The experimental approach to this problem clearly demonstrates the value of such a method and its relative simplicity.

Butcher and Taylor (p. 239) were able to strangulate the vessels at the apices of the pulps of monkeys by relatively great forces in retraction. This should not be interpreted to mean that the relatively light forces used by orthodontists cause this cutting off of vascularity or that this type of strangulation is in any way similar to the "self-strangulation" once believed to be produced by pulpal hyperemia. It is improbable that even the heavy force sometimes applied in intermaxillary wiring for fixation of fractures would be severe enough to result in pulp strangulation.

Kraus (p. 248), investigating the unerupted teeth of pre-Columbian Indians, failed to find confirmation for the statement that the dentino-enamel junction "blueprints" the tooth pattern. The dentino-enamel outline in calcified teeth does not offer indication of the exact outline of the enamel surface. It is suggested that unilaterally active genetic factors may determine the differences between the enamel outline and the dentinal outline.

Bhaskar, Schour, Greep, and Weinmann (p. 257) used parathyroid hormone on *ia* rats in an attempt to correct genetic anomalies. The *ia* rat has a failure of the normal osteoclastic activity and one of the results is distortion and ankylosis of developing teeth. It is extremely interesting that the use of parathormone reduced the incidence in severity of dental anomalies and in many instances permitted the eruption of teeth. The study strongly suggests that the osteoclasts in *ia* rats lack an enzyme which would permit the dissolution of bone matrix, or else that they require a very high threshold of parathormone to produce this enzyme. The possible curative effects of parathyroid hormone, even in the presence of other adverse factors, is clearly demonstrated.

Dreizen, Niedermeier, Reed, and Spies (p. 271) found that ACTH and cortisone influenced the salivary sodium concentration but not salivary potassium concentration. Since the levels of sodium and potassium in saliva did not reflect those in blood, the authors conclude that saliva is more than a filtrate of blood and that the selective concentration of blood salts in saliva is under hormonal as well as neural influence. These significant findings suggest the need for re-examination of some data on salivary composition and its variation. Recently, Sand (*J. App. Physiol.* 4: 66, 1951) presented a similar suggestion of the salivary glands forming or concentrating chemical substances.

Ballantyne, Rae, and Lawford (p. 281) were unable to correlate salivary lactobacillus counts with ammonia production or urease activity in saliva. This entire problem of salivary ammonia-dental caries relationship has been repeatedly revised during the past two decades, but the weight of evidence and opinion appears to indicate a lack of cause and effect relationship.

The effect of metabolic disturbances on the alkaline phosphatase of bones and teeth was investigated by Morse and Greep (p. 284). Neither experimental rickets, hypophysectomy, nor a genetic abnormality in bone growth appeared to profoundly effect the localization of alkaline phosphatase within tissues but in the areas of changes in morphological pattern the distribution pattern varied.

The problem of the hardness and softness of teeth has been debated for many years and a number of individuals have made efforts to study the hardness of enamel by many different methods. It has often been assumed that areas of reduced hardness would also be areas that would be more soluble.

Schwartz and Phillips (p. 293) have investigated this problem using rather precise techniques and found that both the hardness and solubility of enamel varied from tooth to tooth and from area to area within the same tooth. However, no correlation between hardness and solubility was detected. This is an interesting point in consideration of the dissolution of enamel by acids produced intraorally. It also is interesting to consider the relationship of this to the reduced solubility of fluoridated enamel and the fact that fluoridation also appears to produce a harder enamel surface. Apparently, this relationship between hardness and solubility, which is produced by artificial means, does not hold for the inherent condition of the enamel surface.

PANOGRAPHIC RADIOGRAPHY

ROBERT J. NELSEN,* D.D.S., AND JOHN W. KUMPULA

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MODERN methods of diagnosis and treatment of diseases of the teeth and their surrounding structures require that the dentist have a complete series of dental radiographs for each patient.[1] Conventional methods of intraoral radiography necessitate repetitious exposure and processing cycles of small and difficult to handle films. The use of a single strip of film to record the apices of an entire dental arch or a complete bite-wing survey would have obvious advantages over the present methods of dental radiography. These advantages would be most apparent in situations which require x-ray examinations of large numbers of persons such as in the military and public health services.

An apparatus (Fig. 1) has been developed at the University of Washington which will automatically expose a specially shaped film, producing on the single strip, a usable image of the entire dental arch (Fig. 2). This method of radiography has been termed "panographic radiography." It is based upon similar methods described independently by Paatero[2] and Smathers.[3]

OUTLINE OF METHOD

In this method, a narrow beam of x-radiation is directed through the tissues to a lead backed film which is aligned in the mouth by means of a tray type film holder. The full arch exposure is made by the coordinated motion of the object and the narrow beam of x-radiation which causes the film to be exposed somewhat in the same way as the film in a camera equipped with a focal plane shutter.

REQUIREMENTS OF THE APPARATUS

Fig. 3 shows the basic elements of the apparatus. At *A* is the control panel and transformer of the x-ray machine which must have an air-cooled tube *B* permitting the extended operation required in making these exposures. The air-cooled tube is also lighter in weight than the other types of tubes and thereby allows more flexibility and better balance in the operation of the unit. The cone *C* is constructed of a $\frac{3}{32}$ inch lead sheet and is provided with a vertical opening 1 mm. in width and 85 mm. in height. The target film distance is approximately fourteen inches. The stand *D* supports the control panel and an internal element *E* which moves horizontally within the support stand and also rotates about the horizontal axis *XX'*. The horizontal movement of this element permits the source of radiation to be moved linearly for the exposure of the posterior teeth of either side. The rotation of this element provides a

Received for publication, Oct. 25, 1951.

*Now Research Associate for the American Dental Association at the National Bureau of Standards.

means of altering the vertical angulation of the x-ray beam *R* as required during the exposure of the anterior and posterior teeth. The chair is positioned in such a manner that it can rotate about the vertical axis *Z* which intersects the horizontal axis at *O*.

The magnitude of all movements is preselected by various adjustments at the control panel. In function, these movements are selected and controlled by selsens and relays. The unit is hydraulically driven by a pump located separately from the unit to eliminate vibration. The chair is rotated by means of a motor-driven worm gear.

RELATIVE MOVEMENTS OF THE UNIT AND THE PATIENT

Since the movements of the machine and the chair occur about the intersecting horizontal and vertical axes, respectively, and the central beam of radiation rotates about the horizontal axis it is necessary that the patient be

Fig. 1.—Front view of completely automatic unit showing rotating chair and the tube support stand with control panel.

positioned in the chair in such a manner that the center of the arc of the dental arch will be precisely at the intersection of these axes. In other words, the patient must be positioned very accurately to the machine if the correct geometric relation of film-object-radiation is to be maintained during the exposure.

Film trays or holders have been provided to facilitate the placement of the film strip and to stabilize it during the exposure (Fig. 4). The extension

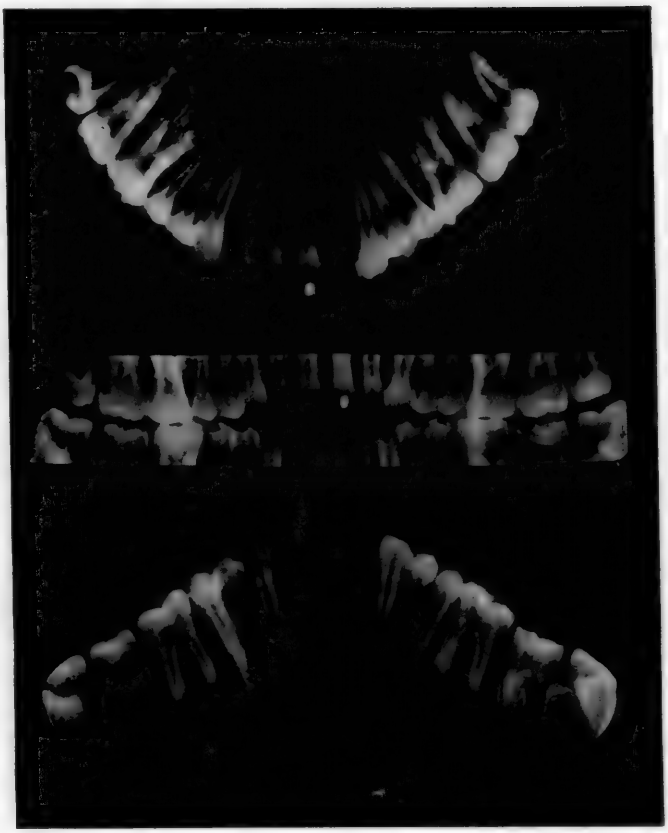

Fig. 2.—Complete periapical and bite-wing radiographs on three films. (Skull)

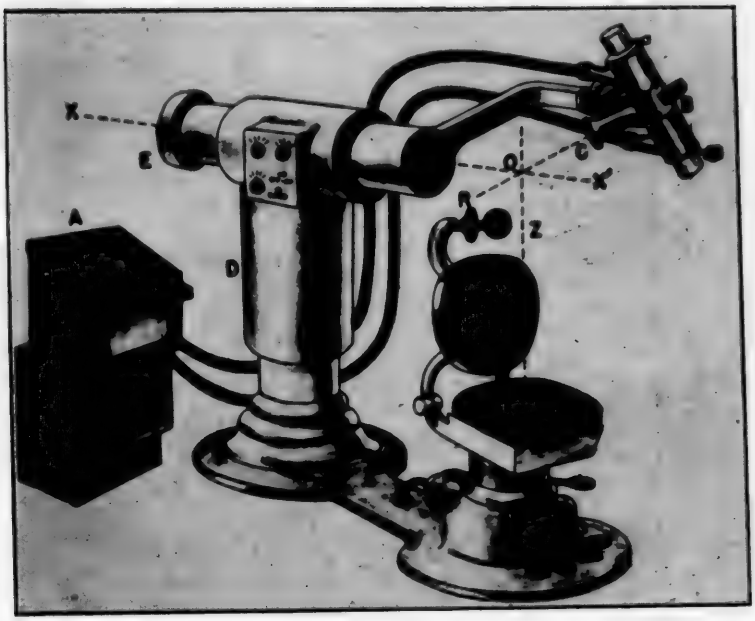

Fig. 3.—Drawing of a panographic x-ray apparatus showing essential elements. *A*, the control panel and transformer of the x-ray machine; *B*, air-cooled tube; *C*, lead-lined cone which passes narrow beam of radiation, central beam of which is represented by *R*; *D*, unit support stand; *E*, horizontal element which moves through *D* and rotates about axis *X*-*X'*; *Z*, axis of chair rotation which intersects axis *X*-*X'* and *R* at *O*.

wings on the film trays function as an indicator of the position of the film in the mouth and serve as a reference in the alignment of the patient in the unit.

Obviously, the dental structures of each patient vary in their arch size, typal form and inclination of the teeth. Prior to the alignment of the patient in the unit, the size and shape of the dental arches must be determined. The method which appears to be the most usable is that in which the patient merely bites on a sheet of wax which has reference lines ruled on it. By using this wax bite as an index, the magnitude of the various movements of the apparatus

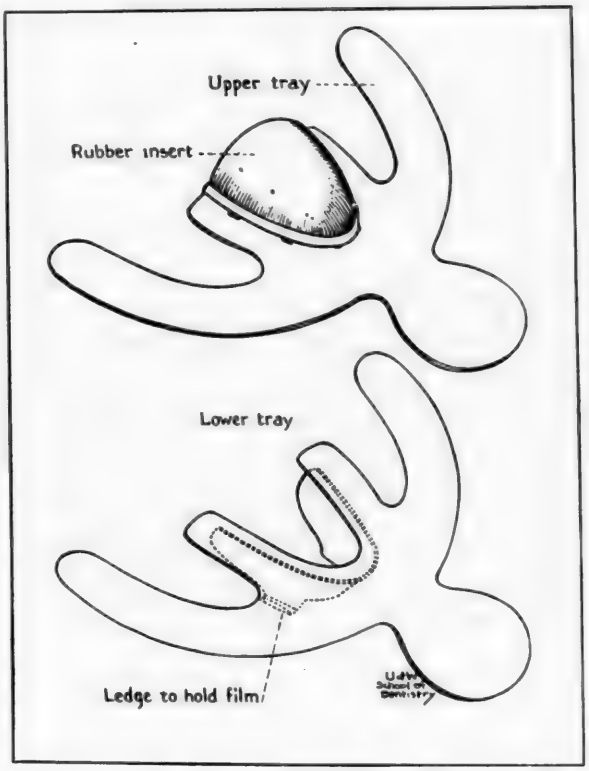

Fig. 4.—Metal film holders which facilitate placement of films and alignment of patient to machine.

is determined and adjustments of the controls made accordingly. These adjustments can be made to vary the length of the horizontal travel of the tube, the extent of the rotation of the chair, and the change in the vertical angulation of the beam of radiation as are required for each patient.

Once the unit has been set for a particular subject, the film placed and the patient properly aligned in the unit, the entire exposure is automatically completed. Fig. 5 shows the slotted tube in its starting position for an ex-

posure of the entire lower jaw, and Fig. 6 illustrates the starting position in the retromolar area and the area exposed as the narrow beam of radiation passes horizontally across the posterior arch segment. The extent of this first movement is determined by the arch size and form. The tube stops its horizontal travel at the point in the arch where the curve of the anterior segment begins. The chair then commences to rotate at this point to expose the curved segment of the arch. When the beam of radiation passes over the anterior teeth, the tube automatically assumes the vertical angulation as determined at the time the unit was set. Fig. 7 shows diagrammatically the manner in which the

Fig. 5.—Film held in place with cotton rolls. Lead-walled cone which passes 1 mm. by 85 mm. vertical beam of radiation Shows machine in motion number one for scanning lower posterior segment of arch. Tube and head move from molars to premolars in a linear direction.

curved surface of the arch is exposed by rotating the patient. Note that the narrow beam of radiation is maintained perpendicular to a tangent at the point of exposure. The tube resumes its original vertical inclination upon the completion of the exposure of the anterior teeth. When the curved portion of the arch has been exposed the chair stops its rotation and the tube resumes its travel in the same horizontal direction to complete the exposure. Fig. 8 shows the patient rotated and the tube beginning its linear travel after reassuming the correct vertical angulation for the posterior teeth.

During all exposues, the same principles of geometric projection apply to these procedures as apply to the conventional methods of dental radiography.

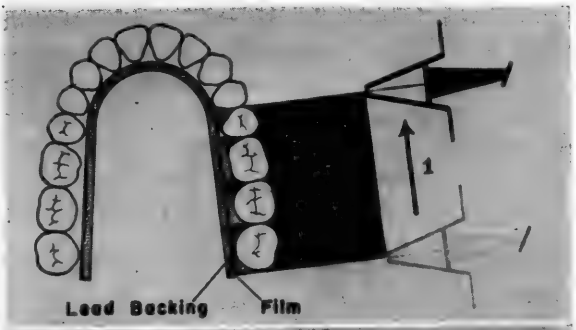

Fig. 6

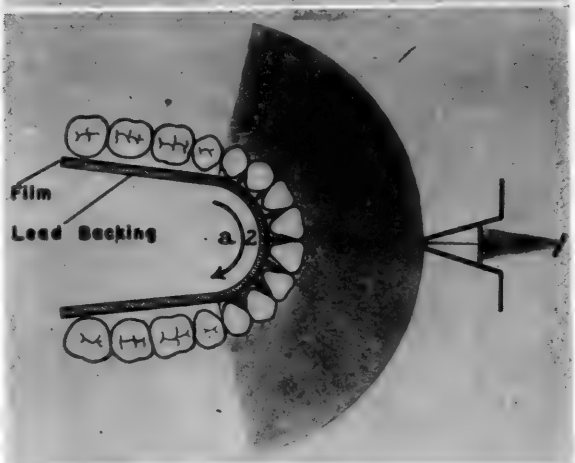

Fig. 7.

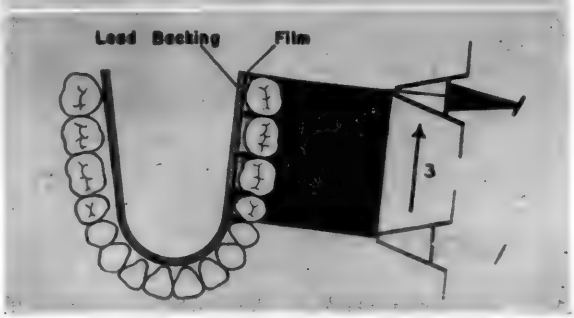

Fig. 8.

Fig. 6.—Schematic view of first motion of x-ray tube from molar to premolar area. The tube stops its horizontal motion and assumes the correct vertical angulation for the anterior segment of the arch.

Fig. 7.—Schematic view of motion number two. Tube assumes proper vertical angulation for anterior segment and then the rotary motion of the patient exposes the anterior teeth. Extent of rotation is dependent upon size and form of the arch.

Fig. 8.—Schematic view of motion number three. The tube resumes the proper angulation for posterior region and moves from premolar to molar region.

PROBABLE APPLICATIONS AND LIMITATIONS OF THIS METHOD

The most practical advantage of this method would be in its application to group surveys such as are required in military dentistry. A second application would be in caries control studies. The apparatus has been designed to permit easy dismantling.

It is believed that panographic radiography will ultimately have a usefulness much as the photofluoroscopic procedures used in the chest survey programs. In the evaluation of the panographic technique it is well to remember that it must be judged as an adjunct to conventional radiography and not as a substitute for it. This innovation and method are by no means in their final form. At present it is questionable whether this system can be applied to all mouths. As there are in conventional radiographic techniques, so also, there are intrinsic geometric limitations to this procedure when it is applied to the extremely square type arch and the very narrow V-shaped mouth.

A simple device for the alignment and stabilization of the patient must be developed along with the most usable assortment of film strip and film tray holders. Not the least problem of the future will be a determination of the total exposure of the patient to radiation under the conditions of this technique.

SUMMARY

An apparatus has been developed which will automatically expose a special single strip film to produce a usable image of the entire dental arch. It is not proposed that such an apparatus and technique will replace conventional intraoral radiography but it will complement it.

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HOMOLOGOUS AND HETEROLOGOUS INTRAOCULAR GROWTH OF TRANSPLANTED TOOTH GERMS

HAROLD S. FLEMING, D.M.D.

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THE several methods recently used by investigators to study the growth and development of the teeth are making serial sections of embryonic jaws,[1, 19, 27] periodically, in certain tooth germ areas; cultivating[12, 13, 21] tooth germs in vitro; observing the effect on continuously growing teeth[4, 6, 8, 30] following the withdrawal of essential elements from the diets of laboratory animals; and subjecting tooth germs to environmental changes by transplanting them to new sites in the same and different animals.[18, 20, 32] The present report is concerned with the in vivo growth of tooth germs under the latter method.

Greene[14, 16] reported that certain embryonic and most malignant tissues have the ability to grow on transfer to the anterior chamber of the eye. He found that adult tissues and benign tumors with the exception of schwannomas, meningiomas, and salivary gland tumors, lack the capacity for growth and differentiation on transplantation to this site. Browning[7] also demonstrated that certain embryonic tissues in mice will survive intraocular transfer to the same and different strains of mice. Since investigators have shown that tooth germs retain their ability for growth and differentiation when cultivated in vitro or grown in vivo, it was felt that tooth germs as embryonic tissues would also grow and differentiate when transplanted to the anterior chamber of the eye. Grobstein[17] reported on the formation of teratoma-like masses on homotransplanting 4 to 5 day embryonic shields of mice from in vitro cultivation to the anterior chamber of the eye where, among other tissue formations, tooth structures were obtained. The present writer has found no reports on the intraocular growth of tooth germs where specific attempts have been made to transplant them to this location. This paper presents such a report.

MATERIALS AND METHODS

The laboratory animals used to receive transplants were mice, guinea pigs, and rabbits. The mice were inbred stock of the dba, C57 Black and C57 Brown strain and C_3H-dba hybrid, first generation. The guinea pigs were open market stock and the rabbits were New Zealand white giants. Animals were selected at random, but young adults were preferred. The sex was always recorded.

Satisfactory material for transplanting was obtained between bud formation and the successive morphologic stages that precede maturation of the enamel and dentin matrices. Calcified material was not desirable, and when present in the transfer caused a severe inflammatory reaction which was followed by suppuration and destruction of the eye.

This material is from a thesis submitted in partial fulfillment of the requirements for the degree of D.Sc.(med.) at the Graduate School of Medicine, University of Pennsylvania.

Received for publication, Sept. 11, 1951. Revised by author, Dec. 3, 1951.

Whole tooth germs of mice, guinea pigs, cats, and rabbits were transplanted homoplastically and heteroplastically to the anterior eye chambers of mice, rabbits, and guinea pigs. Human material, when available, was also transplanted to the eyes of guinea pigs and rabbits and to the axillae and abdomen of mice. In a few instances, enamel epithelium alone, or the dentinal papilla alone, was transplanted.

The dental anlagen of 14 or 15 day mice embryos were found to be satisfactory for these experiments. In rabbit embryos tooth germ tissues obtained between the seventeenth and twenty-sixth day were found to be satisfactory for transplanting. Proliferative stages take longer in guinea pigs and show considerable variance not only between embryos of the same age, but also between littermates, and in the same embryo there may be different levels of tooth development from one side of the jaw to the other. These findings concur with those of Harman and Smith.[19] Dental anlagen or tooth germs of guinea pigs were used when the embryos were between 15 and 30 days of age. Tooth germs of the teeth of 30 day cat embryos were found to be at the bell stage of development, and this material proved to be satisfactory for transplantation. Human fetuses or embryos were dated from the time of the last menstruation of the mother. Four to 5 month fetuses were most satisfactory. In older fetuses suitable tooth germ material for transplanting was generally taken from the deciduous canine or first molar tooth germs.

Tooth germs were aseptically removed from the jaws of embryos or fetuses. This material was then transferred to a sterile covered Petri dish, where it was kept moist with serum from the embryo or sterile normal saline, until ready for use. When there was a question of contamination, aqueous penicillin, in a concentration of 25,000 units per c.c., was dropped over the material. This solution appeared to interfere with subsequent growth and differentiation of the transplant, so its use was avoided whenever possible. Usually, when the size permitted, the entire dental follicle was transplanted to the animals selected as hosts. At other times, selected portions of tooth germs were explanted. From each group of series of transfers, representative tooth germ material of the particular type used was saved as a histological control. Half of a jaw with the tooth germs in situ was routinely sectioned and stained, while the tooth germs were dissected from the other half and transplanted.

The technique employed for transplanting was that perfected by Greene.[15] Tissue was carried to the eye in specially designed No. 16 and No. 20 trocars. Best results were obtained by making the incision, at the limbus of the cornea, just large enough to receive the trocar. Large incisions or oversized pieces of tissue invited failure because these factors enabled the transplant to escape from the anterior chamber. After insertion, the material was manipulated within the anterior chamber to the growth site at the junction of the iris and cornea.

General anesthesia was used for mice and the dose was administered subcutaneously—a .01 c.c. of Nembutal, diluted 1:10, being given for every gram of body weight. Rabbits and guinea pigs were usually restrained and local

TABLE I

HOMOTRANSPLANTS—SUMMARY OF BIOLOGICAL BEHAVIOR

SPECIES	STRAIN	NO.	SEX	AGE DONOR EMBRYO	DONOR TYPE	AGE OF HOSTS	TRANSFER SITE	FATE OF HOSTS	GROWTH PERIOD	FATE OF TRANSFERS
Rabbits	New Zea. White	17	15m. 2f.	18 to 21 days	same	adult young	both eyes	all lived	7-31 days and 1 for 58 days	16 growth 1 scar tissue
Guinea Pigs	open stock	61	31m. 30f.	15 to 30 days	same	adult young	one eye	21 discards death or infection	6-30 days and sometimes longer 6 for as long as 300 days‡	26 growth 8 scar tissue & bone 6 resorption
Mice	C57Brown C57Black dba hybrid	71	36m. 35f.	14 days	dba & hybrid	adults and young adults	one eye	20* died 51 lived	7-28 days	resorption only 26 cases had material to section
Mice	C57Black C57Brown	49	25m. 24f.	14 days	as above	as above	19 axillae 18 abdomen 12 brain	6† died rest lived	7-50 days	growth in all except 5 animals

*12 died—failed to survive the anesthesia and the operation, 8 were found dead in the cages and were thrown out.

†3 failed to survive the brain operation and 3 were found dead in the cages. Resorption became increasingly evident after the third week in all eye transfers.

‡These showed continued growth after 28 days and were kept for periods up to 300 days. An example is seen in *Fig. 13*.

anesthesia of the eye was obtained by applying a few drops of a 5 per cent cocaine solution (previously autoclaved) several minutes before the incision was made.

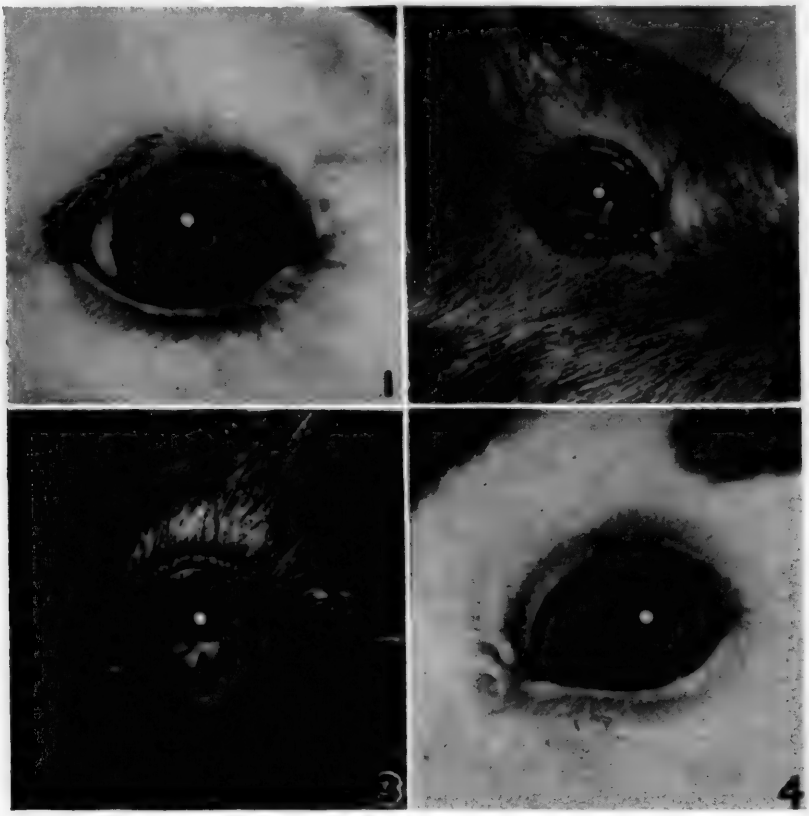

Fig. 1.—Eye of a rabbit, after bearing a transplant of a rabbit tooth germ for 18 days. Fig. 17 shows a section of this transfer after the animal was sacrificed.

Fig. 2.—Eye of a guinea pig after bearing a transplant of a guinea pig tooth germ for 14 days. Animal was then sacrificed and Fig. 11 shows section of transfer.

Fig. 3.—Eye of a guinea pig after bearing a transplant of human tooth germ for 20 days. Animal was sacrificed later and Fig. 27 shows section of recovered transplant.

Fig. 4.—Eye of a rabbit bearing transplant of human tooth germ material for 20 days. Animal was then sacrificed and Fig. 26 shows a section of recovered transplant.

No special care was given to the animals after they received the transplant. They were examined daily so that the biologic behavior of the explant could be followed. Following the transplantation, the cornea became clouded, obscuring the graft. As the cornea cleared up, outlines of the transplants became definite and after 14 days the formations of crowns of teeth were usually visualized intraocularly in guinea pigs and rabbits.

Figs. 1 to 4 show pictures of the eyes of guinea pigs and rabbits that have received transplants of tooth germs. Figs. 1 and 2 are of homotransplants and Figs. 3 and 4 are of heterotransplants.

TABLE II

HETEROTRANSPLANTS—SUMMARY OF BIOLOGICAL BEHAVIOR

DONOR	HOSTS	SITES	FATE OF HOSTS	DURATION OF GROWTH	FATE OF TRANSFERS
21 day rabbit embryos	5 G.P.m. 1 G.P.f.	one eye	all lived	11-21 days	2 resorbed 1 scar tissue 1 bone and cartilage 2 growth and differentiation
25 day rabbit embryos	3 G.P.m. 4 G.P.f.	one eye	1 died	8-21 days	2 resorbed 1 bone and cartilage 3 growth and differentiation
18 day rabbit embryos	3 G.P.m. 3 G.P.f.	one eye	all lived	14-26 days	1 resorbed 1 scar tissue 1 bone and cartilage 3 growth and differentiation
30 day cat embryos	3 rabbits 3 G.P.m. 3 G.P.f.	both eyes one eye	all lived 1 lost	7-28 days	2 resorbed 6 growth and differentiation
4 mo. human embryo	6 G.P.m. 4 G.P.f. 2 mice	one eye axillae	2 G.P. died	10-52 days	4 resorbed 1 scar tissue 3 growth and differentiation both mice showed growth
5 mo. human fetus	2 rabbits 7 G.P.m. 3 G.P.f.	one eye	all lived	10-28 days 1 for 120 days*	5 resorbed (4 G.P. and 1 rabbit) 3 scar tissue 4 growth and differentiation
5 mo. human fetus	2 rabbits 5 G.P.m. 5 G.P.f.	both eyes one eye	all lived 1 died and 1 lost	10-28 days 1 for 160 days*	3 resorbed (2 G.P. and 1 rabbit) 2 scar tissue 1 bone 4 growth and differentiation
newborn human	4 G.P.m. 2 G.P.f.	one eye	1 died	10-30 days	2 resorbed 1 scar tissue 2 growth and differentiation

A total of 68 animals received eye transplants from alien species. Six were discarded because of death and one was lost. In the remaining 61 animals growth and differentiation were obtained in 27 cases.

*Transplants kept for longer periods.

Several days were needed for a transplant to establish itself in the anterior chamber of the eye. For this reason no animals were sacrificed until at least 6 days after the operation. The recovered graft was fixed in Carnoy's solution. If calcified material was present in the recovered transplant it was decalcified before sectioning and staining. Sections were usually cut at 5 μ, but if a considerable amount of tooth structure was present, thickness of the cuts was sometimes increased to 10 μ. Hemotoxylin and eosin were used to stain the sections. A representative result from each series of transfers is reported on and discussed in the text of this paper.

Excluding homotransplants to the eyes of mice, successful "takes" were observed in approximately 50 per cent of 146 cases. This included 28 guinea pigs that failed to survive or died soon after the operative procedure. Excluding discarded animals, positive results were found in 57 per cent of the cases. Eye transplants of mice were not included because the results were unfavorable (Tables I and II).

The words homologous and heterologous are employed with the same connotation that Greene[14] uses them; thus, the word homologous designates transplants between unrelated animals of the same strain or species and the word heterologous designates transplants between animals of different species.

EXPERIMENTS IN HOMOLOGOUS TRANSPLANTS

Mouse Homotransplants.—Seven series of eye transfers were made to inbred strains of C57 Browns and Blacks, to dba's and to first generation hybrids from C_3H and dba strains. Approximately 70 animals were used, with 8 to 12 of a particular strain forming each series. Tooth germs were obtained from 14 day hybrid and dba embryos. From 7 to 28 days was the period allowed for growth.

Following the initial period of activity, which lasted from 4 to 7 days, these transplants usually began rapid resorption, so that by the fourteenth day little remained of the original transfer. In several cases, transfers persisted for longer periods and material recovered after 21 to 23 days showed, on sectioning, clusters of epithelial cells with intraepidermoid keratinization. The connective tissue present was of a collagenous nature. Infrequently bone and islands of osteodentin were found. Ameloblasts and odontoblasts always lost their characteristic columnar pattern and disappeared.

Additional series of tooth germs were homotransplanted to the axillae, abdomen and brains of mice in order to compare the biological behavior of explants to these sites with eye transfers. Embryonic lung tissue and limb buds were also homotransplanted to the eyes of mice to cross-check the technique. There was growth and differentiation of these tooth germs when transplanted to other locations. Eye transplants of other prenatal tissues grew and differentiated.

Fig. 5 shows a transplant in the eye of a C57 Black male after 21 days. A 14 day C_3H-dba hybrid embryo was the source of the tissue. A layer of large squamous cells may be seen along the border of wavy bundles of pale staining collagenous material that has incorporated in it scattered dark stain-

ing cells. On the inside of this layer there are cords of cells, some of which are heavily pigmented. These structures represent the corneal epithelium, layers of the cornea, and regenerating pigmented layers of the iris. Within this area, which represents the anterior eye chamber, there are eosinophilic-stained regions that have included cells and are bordered on one side by cells with large oval nuclei. Along other sides occasional giant cells are seen.

Fig. 6 shows the recovered transplant of a 14 day tooth germ from a C_3H-dba hybrid embryo grown in the brain of a C57 Brown female for 40 days. Two developing teeth are seen which have partly calcified enamel and dentin. Odontoblasts are identified around the peripheral border of the pulpal area of each tooth. Histologically there is an abundant blood supply and large vascular spaces are seen within the pulpal areas. The enamel epithelium has apparently finished its activity. A large epithelial pearl lies near these structures. There is a large amount of bone, but no cartilage is seen. A number of small round cells (lymphocytes) are seen near by, indicating a cellular attack by the host.

Fig. 7 illustrates a recovered axillary transplant. The dental anlage was obtained from a 14 day C_3H-dba hybrid embryo and the host was a C57 Brown female. The growth period was 20 days. In this illustration the hard structure of the tooth is represented by a continuous layer of dark pink-staining homogeneous material that is surrounded by connective tissue cells. On the inside border of the thickest side of this tooth structure there is a row of pale staining cells with large oval nuclei. Long processes from these cells enter the thickened material and continue in its entire thickness. These cells are odontoblasts and they are associated with the crown portion of this tooth. A very pale thin layer of partly calcified enamel rods is on the surface of this crown area. The connective tissue of the pulpal area is loosely arranged and some blood vessels are seen in it.

Fig. 8 shows a transverse longitudinal section of the anlage of a mouse molar in situ which was taken from a 14 day C_3H-dba hybrid embryo.

Guinea Pig Homotransplants.—Six series of guinea pig homotransplants were made. The material transplanted came from embryos of between 15 and 30 days. From 6 to 30 days was the usual growth period, but in some cases, explants remained in the eye chamber for as long as 300 days. In each series there were from 8 to 12 animals, generally evenly divided as to sex. For this experiment a total of 61 guinea pigs were used, of which 21 were discarded because of death or infection before the fate of the transfer was determined. of the 40 guinea pigs saved, 26 showed growth and differentiation of transplanted tooth germs; and in 14, scar tissue was present when there was not

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Key to Symbols:

A, Ameloblasts
B, Bone
C, Cyst
D, Dentin
E, Enamel
G, Giant Cells
I, Iris-pigmented layer
K, Keratinization
O, Odontoblasts
P, Pearl Formation
Br, Brains
BV, Blood Vessel
CE, Corneal Epithelium
Co, Cornea
DEp, Degenerated Epithelial Cells
EP, Enamel Pulp
ER, Enamel Rod
DM, Descemet's Membrane
DT, Dentinal Tubules
Ly, Lymphocytes
OD, Osteodentin
OS, Osteoclasts
SI, Stratum Intermedium
SR, Stellate Reticulum
TF, Tomes' Fibers
TP, Tomes' Processes

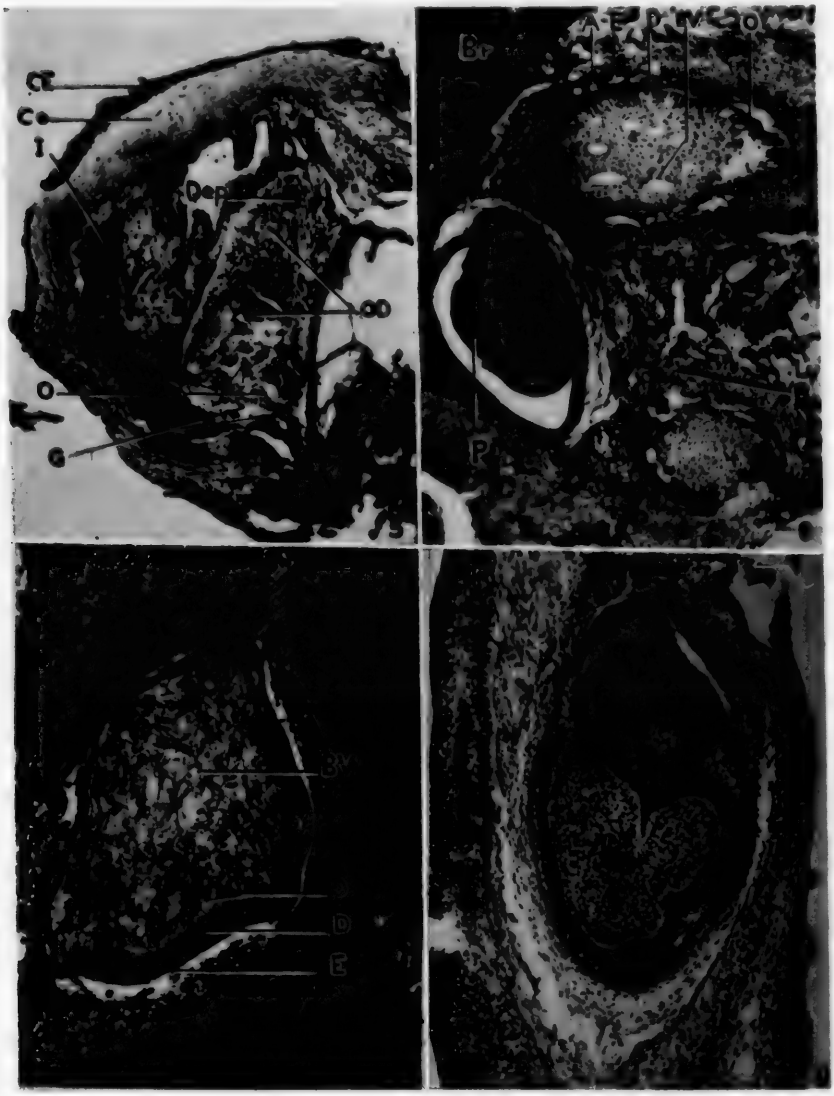

Fig. 5.—Section through the eye of a C57 Black mouse after 21 days' growth of a tooth germ taken from a 14 day C3H–dba hybrid embryo. Original magnification ×140.

Fig. 6.—Recovered brain transplant of a tooth germ from a 14 day C3H–dba hybrid embryo, grown in a C57 Brown female for 40 days. Original magnification ×125.

Fig. 7.—Section of a recovered axillary transplant. The tooth germ was obtained from a C3H–dba hybrid embryo of 14 days and was transferred to the axilla of a C57 Brown mouse where it was allowed to remain for 20 days before the animal was sacrificed. Original magnification ×260.

Fig. 8.—Longitudinal section through a molar tooth bud of a 14 day C3H–dba hybrid mouse embryo which is at cap stage of development. Original magnification ×130.

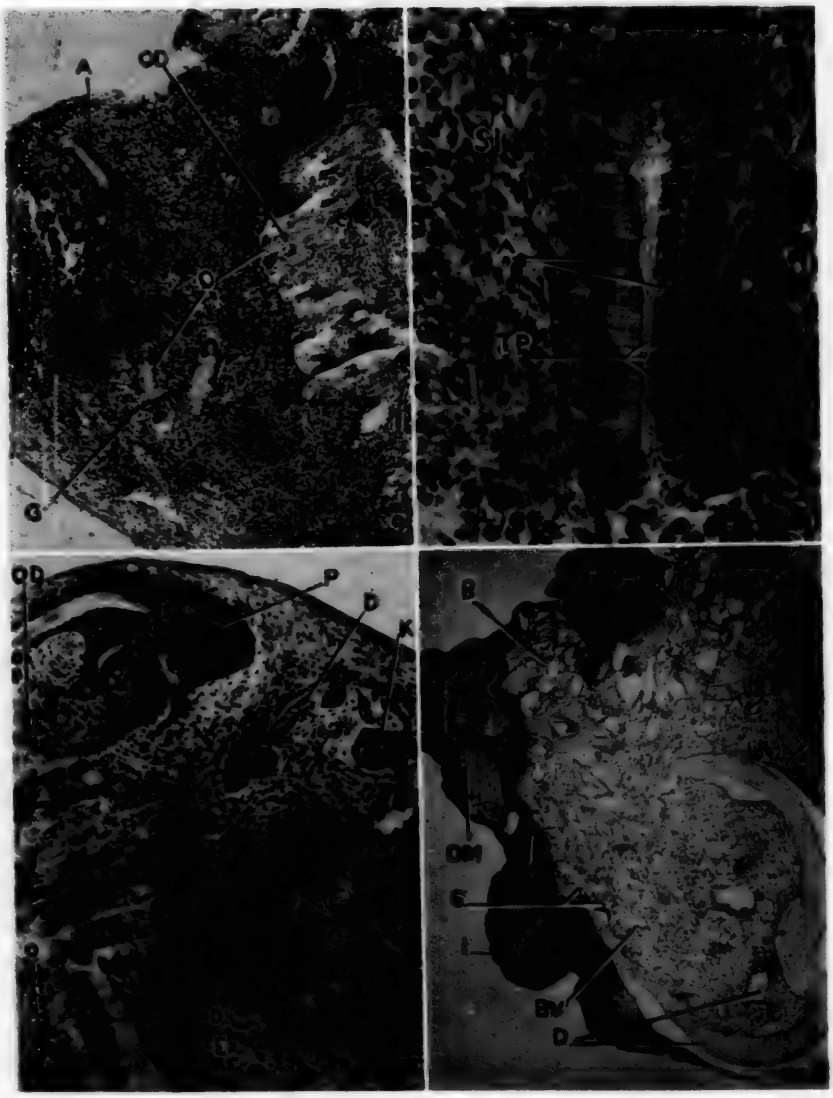

Fig. 9.—Section of transplant recovered from eye of guinea pig after 21 days' growth. Tooth germ was from a 30 day guinea pig embryo. Original magnification ×120.

Fig. 10.—Higher magnification of the upper left corner of Fig. 9 at *A*. Original magnification ×500.

Fig. 11.—Section of transplant removed from eye of guinea pig after 14 days' growth. Source of the tooth germ was a 23 day guinea pig embryo. This picture was taken at the junction of the inner and outer enamel epithelium, from which the epithelial root sheath of Hertwig forms. Original magnification ×135.

Fig. 12.—Section of a guinea pig's eye with a transplanted tooth after 28 days' growth. The anlage came from a 23 day guinea pig embryo. Original magnification ×55.

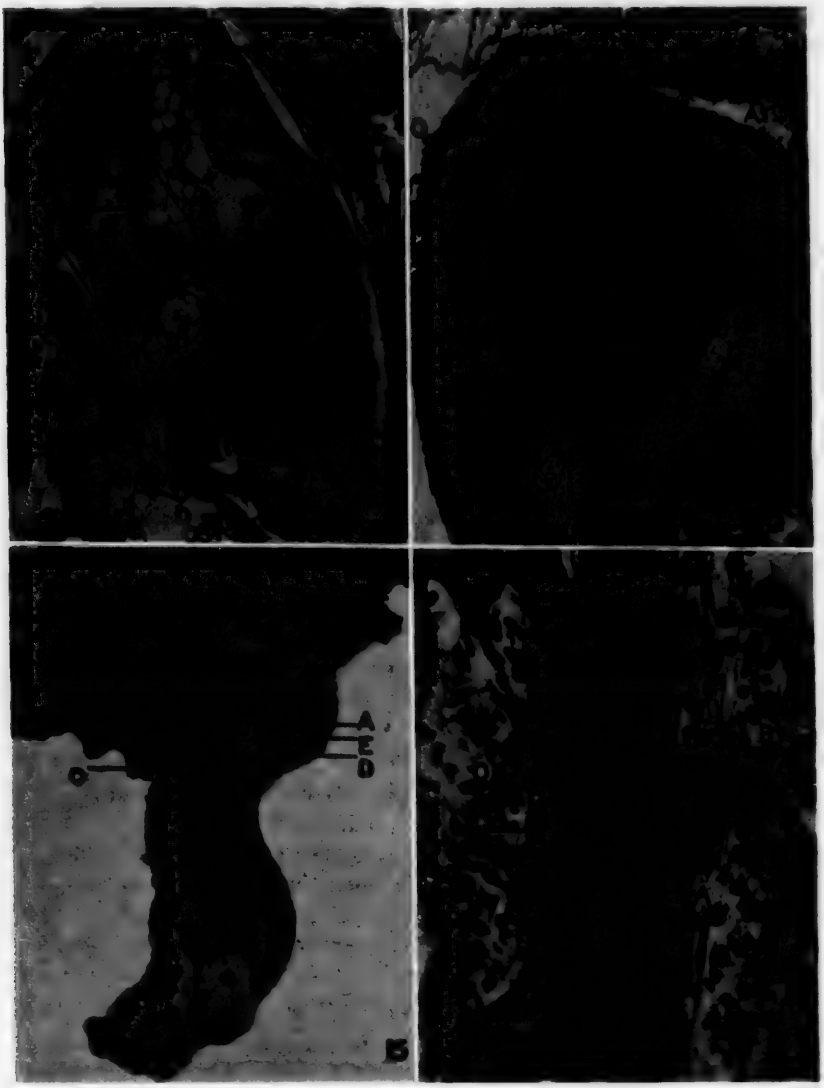

Fig. 13.—Section through the eye of a guinea pig after bearing a transplanted tooth germ from a 23 day guinea pig embryo for 174 days. Original magnification ×65.

Fig. 14.—Horizontal section through a molar tooth of a 23 day guinea pig embryo. This is at bell stage of development. Original magnification ×55.

Fig. 15.—Section of recovered homotransplant of rabbit tooth germs after 12 days' growth in the eye of a New Zealand white giant. The material was from a 21 day rabbit embryo. Original magnification ×47.

Fig. 16.—Higher power of Fig. 14 of upper tooth in area of columnar arrangement of ameloblasts and odontoblasts. Original magnification ×500.

absorption of the transplant. Over long peroids the hard structures of the teeth would always tend to lose their characteristic appearance and were replaced by metaplastic bone.

Fig. 9 represents the recovered transplant from a 30 day embryo, grown in the eye of a male guinea pig for 21 days. This section is shown because it illustrates the continued activity of ameloblasts and odontoblasts when separated from each other. In parts of this picture, there are islands of homogeneous eosinophilic staining material associated with disoriented odontoblasts. Giant cells are found near this osteodentin.

Fig. 10 is a higher magnification of *A* in Fig. 9. Tall columnar cells with pale staining nuclei are seen. Their arrangement and the processes extending from them into the lumen which they border identify these cells as ameloblasts which are forming the pre-enamel matrix.

Fig. 11 represents the recovered graft of a tooth germ from a 23 day guinea pig embryo grown in the eye of a male guinea pig for 14 days. In several places intra-epidermoid keratinization is seen in direct relationship to isolated dentin formation. It is possible that these epithelial cells are the degenerated remnants of the inner or outer enamel epithelium. An area of eosinophilic staining material has a number of cells included in it and is directly continuous with the dentin formation of the tooth. These included cells are osteocytes and this material is undergoing osteoclastic changes. There is additional dentin in island formation with odontoblasts arranged along one border. Multinucleated giant cells are seen near these islands of dentin. Resorptive processes in teeth and bone are characterized by the presence of these cells whose origin is either from capillaries in connective tissue or from the connective tissue itself.[5] Plump fusiform cells forming capillary-like structures are also observed.

Fig. 12 shows a tooth germ obtained from a 23 day embryo and grown in a male guinea pig's eye for 28 days. This illustration presents a section through a molar tooth. Dentin remains and the enamel has been lost by decalcification. Tomes' fibers are seen in the dentin tubules and bone formation borders one side of this tooth structure. Hyperchromatic cells of the pigmented layer of the iris and Descemet's membrane are seen. Thin-walled vessels filled with red blood cells are present in the loose connective tissue of the pulpal area. Giant cells near the base of the dentin indicate the beginning of osteoclastic resorption.

Fig. 13 represents the recovered transplant of a 23 day tooth germ that remained in a male guinea pig's eye for 174 days. The anterior chamber was almost entirely filled with areas of eosinophilic staining material having numerous included cells. This osteoid formation is intimately associated with material that stains deeper on the periphery and lighter toward the center. Included cells are occasionally found along the light-stained inner border. Regularly arranged odontoblast-like cells with large pale nuclei are seen in places along this inner border. Fanlike collagenous fibers (Korff's) are seen in the deeper staining peripheral part and identify this material as partly calci-

fied dentin. In places along the outside of this dentin, near the inner border of the corneal wall, are small sections of thin, pale, and crystalline appearing material which is the remains of a layer of enamel. Throughout this entire area are areolar-like spaces closely associated with numerous vascular spaces and thin- and thick-walled vessels filled with red blood cells. A small area of the connective tissue in the center is of stellate character but along the lower right periphery of the figure it is of a collagenous nature.

Fig. 14 shows a transverse section through the cusp of a molar tooth germ from a 23 day guinea pig embryo.

Rabbit Homotransplants.—Young adult New Zealand white giants were used to receive dental anlagen from rabbit embryos of a known age. Two series of transfers were made with 18 day embryos and 2 additional series were made with 21 day embryos. From 3 to 5 animals were in each series. Seventeen rabbits were used for this experiment (15 males and 2 females). Transfers were always made to both eyes and, with one exception, were allowed to remain in the anterior chambers from 7 to 31 days. None of the animals died and the only case that failed to show positive results was one in which the transplant remained in place for 58 days. In this case only scar tissue remained.

Fig. 15 shows a 12 day intraocular growth of a transplanted tooth germ taken from a 21 day embryo. The host was a male rabbit. The area above that shown in the figure has several epidermoid inclusion cysts. Some of the epithelium has keratinized and there are also several epithelial pearls. Near these cystic areas are numbers of lymphocytes. Numerous thin-walled blood vessels, filled with red blood cells, are observed throughout the entire section. Connected to this main body of tissue is the peninsula shown in the figure containing 2 teeth which are in the appositional stage of development. Hexagonal shaped Tomes' processes are observed at one cusp tip, where enamel rods are being formed.

Fig. 16 is a higher power of the area in Fig. 15 at D and O. Considerable newly formed tooth structure is present with ameloblasts and odontoblasts in normal relationship.

Fig. 17 is from the 21 day intraocular growth of a tooth germ transplanted from a 21 day rabbit embryo to a male rabbit. A large amount of dentin formation was seen as well as Descemet's membrane and regenerating corneal tissue. A few osteoclasts are found near one side of the dentin formation indicating osteoclastic changes or reconstruction. A number of lymphocytes are observed in the connective tissue adjacent to the dentin. Similar structural changes of dentin were observed in other instances in these experiments. Other examples of this are illustrated in Figs. 11, 13 and 18.

Fig. 18 represents the 30 day intraocular growth of a tooth germ transplanted from a 21 day embryo to a female rabbit. A great amount of bone formation is present. Large clusters of epithelial cells are also seen, some of which show a central degeneration or necrosis. A cystic area filled with desquamated epithelial cells is located adjacent to a large island of dentin and

osteodentin. A number of giant cells arranged along the right border of this osteodentin indicate resorptive changes. In these experiments, similar cystic areas were found in other instances.

Fig. 19 shows a transverse section of a mandibular tooth germ in situ, from a 21 day rabbit embryo. The development is at the cap stage. Tooth germs from the other half of this jaw and from the jaws of littermates of this embryo were transplanted.

EXPERIMENTS IN HETEROLOGOUS TRANSPLANTS

Rabbit to Guinea Pig Transplants.—In this experiment 3 series of transfers were made to the eyes of guinea pigs with 6 to 8 animals in each series. Rabbit embryos of 18, 21, and 25 days of age supplied the dental anlagen. One eye in each animal received the transplant which was usually grown there from 18 to 26 days before recovery and sectioning. Eleven males and 8 females were used for this experiment. Three animals died, and of the remainder, 13 had evidences of the growth and development of transplanted tooth germs. Regression and resorption of transplants were directly proportional to the length of time they remained in the anterior eye chamber. After the third week transplants became progressively less vascular in appearance and smaller in size.

Fig. 20 shows the recovered transplant of the anlage of a tooth germ obtained from a 21 day rabbit embryo and grown in the eye of a male guinea pig for 8 days. A large horseshoe-shaped area of eosin-stained material, identified as dentin bordered by a thin layer of enamel, was seen. This is surrounded by squamous epithelium and dense connective tissue. On the right border are seen heavily pigmented cells of the iris. Farther to the right there is a dense infiltration of lymphocytes. Inside the tooth formation there are a number of thin- and thick-walled vessels filled with red blood cells. Connective tissue in this area is of a loose stellate type and along its periphery odontoblasts are observed with long fibers extending from them into the dentin. In places there are several included cells. The presence of giant cells near by indicates osteoclastic activity.

Cat to Guinea Pig and Cat to Rabbit Transplants.—There were two series of eye transplants in this experiment: one was made from cats to rabbits, the other from cats to guinea pigs. In one series, 3 male rabbits received transplants of cat dental anlagen to both eyes. In the other series, 3 female and 3 male guinea pigs received transplants of this same tooth germ material to their right eyes. In both groups, 2 rabbits and 4 guinea pigs had positive evidences of the growth and differentiation of the transplanted tooth germs.

Fig. 21 represents a cat to rabbit transplant. In this case, the tooth germ element was allowed to grow for 21 days before the animal was sacrificed. A curved band of tooth structure, cut in the same plane as the dentinal tubules, dominates this picture. Along the peripheral border there are small fragments of crystalline appearing material identified as enamel. Bordering the inside of this structure are odontoblasts and from some of these cells, Tomes' fibers are seen entering the dentinal tubules. Near by, but not seen in this picture, are small amounts of bone and cartilage.

Fig. 22 shows tooth germs in the mandible of a 30 day cat embryo.

Human to Guinea Pig and Human to Rabbit Transplants.—The jaws of human embryos or fetuses were dissected and tooth germs were removed from one-half of the maxilla and mandible. These were transplanted to the experimental animals. The remaining half of the jaws, after sectioning and staining, served as a histological control. Each fetus or embryo used, constituted a series. Tooth germs from a 4 month human embryo were transplanted to 6 male and 4 female guinea pigs. From this same embryo tooth germs were transplanted to the axillae of dba mice. Transplants from two 5 month fetuses were made to the eyes of guinea pigs and rabbits. Twelve male and 8 female guinea pigs received these transplants. In addition, 4 male rabbits also received transplants from these 5 month fetuses. In another series enamel epithelium from the canines of a newborn child was transplanted. Four male and 2 female guinea pigs received these transfers. The large size of the deciduous molar tooth germs in the older fetuses made it necessary to divide them before they could be transferred, but this did not have any effect on subsequent growth. Human material usually reacted favorably to intraocular transfer, but sometimes these transplants failed to grow and rapidly became absorbed. The usual time that these transfers remained in the anterior eye chamber was from 10 to 28 days. However, in some instances they were allowed to remain for as long as 160 days.

Fig. 23 is of the recovered transplant of a lower right deciduous canine transplant that remained in the anterior eye chamber of a female guinea pig for 52 days. A 4 month human fetus supplied the material. This picture illustrates a deveoping tooth. The dark pink-staining homogeneous area of the cusp is composed of partly calcified dentin. Flattened epithelial cells lie against this formation and at the left side there is a cluster of epithelial cells identified as ameloblasts. Odontoblasts grouped along the inside, increase in number toward the extremities of this dentin formation with which they are closely associated. On either side, where dentin formation terminates, are clumps of darkly stained epithelial cells precursors of the sheath of Hertwig. Except for a clump of ameloblasts and enamel at A and E aplasia of enamel formation is significant.

Fig. 24 represents the recovered transplant of a human lower second incisor tooth germ taken from the mandible of a 4 month fetus and grown in the anterior chamber for 90 days. Tooth structure and the various layers of cells associated with tooth development dominate this picture. The cells of the ameloblastic layer are short and cuboidal and in places there are strands of keratin-like material. Bits of enamel fragments are seen in places, but most of the enamel has been lost by decalcification. The dentin is cut in a way to show its tubules and along the periphery of the pulpal area odontoblasts are seen. In places the stellate reticulum of the enamel pulp is very narrow or practically nonexistent, but the outer enamel epithelium joins the ameloblastic layer in the region of the future epithelial root sheath of Hertwig. The pulpal and surrounding areas of this figure are extremely vascular. Dense connective tissue surrounds the entire area. After 90 days there has not yet been an attempt, by the host, to revise this tooth structure.

Fig. 17.—Section of recovered homotransplant of a rabbit tooth germ after 21 days in the eye of a New Zealand white giant. The material was from a 21 day rabbit embryo. Fig. 1 is of the transplant in the eye. Original magnification ×35.

Fig. 18.—Section of recovered homotransplant of rabbit tooth germ after 30 days in the eye of a New Zealand white giant. The material was from a 21 day rabbit embryo. Original magnification ×50.

Fig. 19.—Section of a mandibular tooth germ from a 21 day rabbit embryo. Original magnification ×52.

Fig. 20.—Section through a guinea pig's eye bearing a developing tooth obtained from a 21 day rabbit embryo. This animal was killed after 8 days. Original magnification ×145.

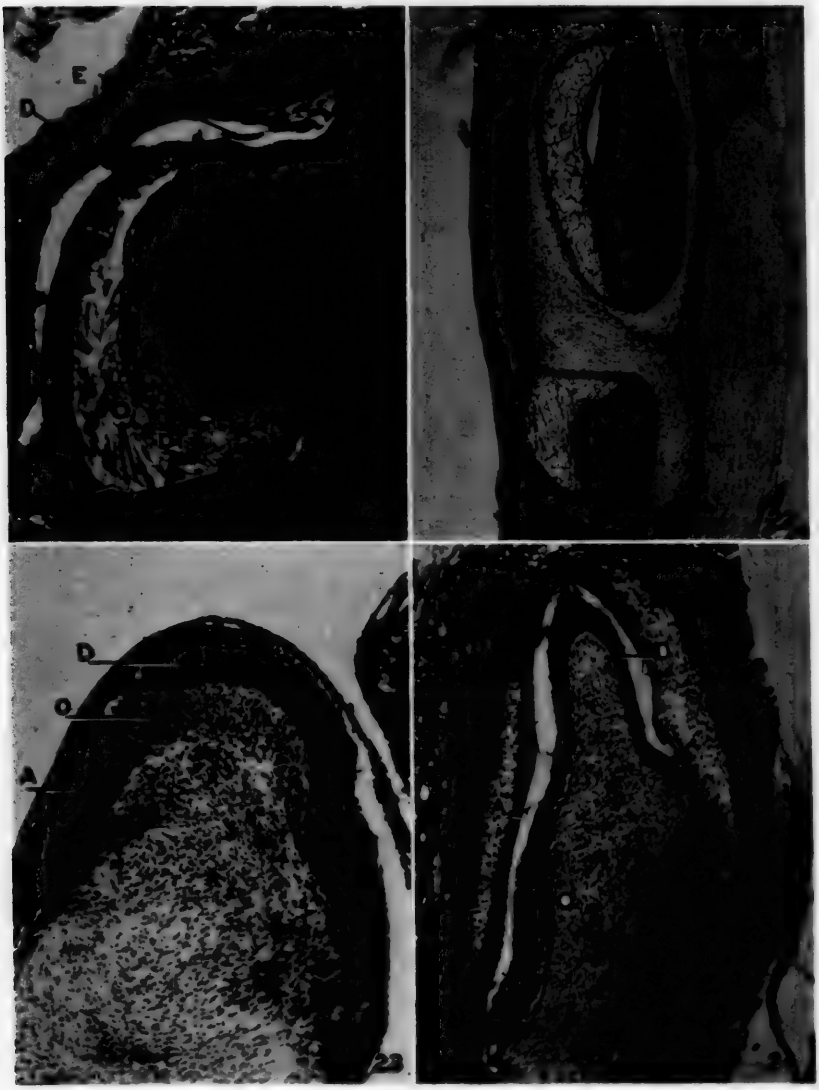

Fig. 21.—Section through the eye of a rabbit bearing a transplant of a tooth germ from a cat embryo. Growth was maintained for 21 days. Original magnification ×280.

Fig. 22.—Section represents mandibular tooth germs from a 30 day cat embryo. Original magnification ×130.

Fig. 23.—Section of a guinea pig's eye after bearing a transipant of a human tooth germ for 52 days. A 4 month embryo supplied the material. Pregnancy of the animal was discovered several weeks after the tissue was transplanted. Original magnification ×40.

Fig. 24.—A 4 month human fetus supplied this tooth germ, a lower second incisor, and it remained in the anterior eye chamber of a male guinea pig for 90 days. Original magnification ×165.

Fig. 25 shows a deciduous mandibular canine tooth germ from a 4 month human fetus. The tooth germ from the opposite side of the mandible was transplanted and is represented in Fig. 23. Ameloblasts are in columnar formation and odontoblasts are recognizable at the tip of future cusp.

Fig. 26 represents the recovered transplant of a lower left deciduous canine obtained from a 5 month fetus and transferred to the anterior eye chamber of a male rabbit. It was grown there for 20 days. In the center of this picture there is a large area of dentin. Some heavily pigmented cells of the iris are near by and to one side there is a portion of Descemet's membrane. In several places there is a focal infiltration of round cells. Several small clumps of degenerated epithelial cells lie near the dentin formation. Various sized vessels filled with red blood cells are located close to the dentin. Along one border there are a number of large multinucleated giant cells. At the opposite border and close to the dentin are odontoblasts. Dark-staining Tomes' fibers are located within the tubules in the dentin.

Fig. 27 shows a transplant recovered from the eye of a male guinea pig after 24 days' growth. It was obtained from the same 5 month fetus that supplied the material shown in Fig. 26. Surrounded by the stellate reticulum there is an organization of ameloblasts which are forming the pre-enamel matrix. Odontoblasts and dentin are also observed. Concentrically arranged connective tissue surrounds this area which has been infiltrated by small round cells. Many vessels filled with red blood cells are also seen. The pigmented layer of the iris is seen in parts of this section.

Fig. 28 is a higher magnification of an area from Fig. 27 inside cusp tip at *A*. It shows enamel prisms, ameloblasts, enamel and dentin.

Fig. 29 illustrates the result of 25 days' growth of a transplanted layer of ameloblasts and odontoblasts. This material was obtained from the deciduous lower canine tooth follicle of a newborn child and it was grafted to the anterior eye chamber of a female guinea pig. In the center of this illustration there is a heavy band of partly matured dentin. Odontoblasts and ameloblasts follow the course of this structure. In places among the dense layer of epithelial cells that surround this area mitotic figures are observed indicating that after 25 days this transplanted tissue is still active and regenerating. Some odontoblasts found near parts of this formation lack a definite arrangement.

Fig. 30 shows several strands of tissue similar to that transplanted to give the result shown in Fig. 29. Ameloblasts in this picture are represented by continuous bands of darker staining material and odontoblasts are arranged opposite them.

DISCUSSION

The transplantation of whole tooth germs or their elements to the anterior chamber of the eye from man to lower animals and between lower animals was successfully carried out in the present experiments and may offer a new approach to the study of their growth and development. Whole tooth germs, when transplanted to the anterior eye chamber, retained the morphology of developing teeth better at this site than at other transplantation sites.

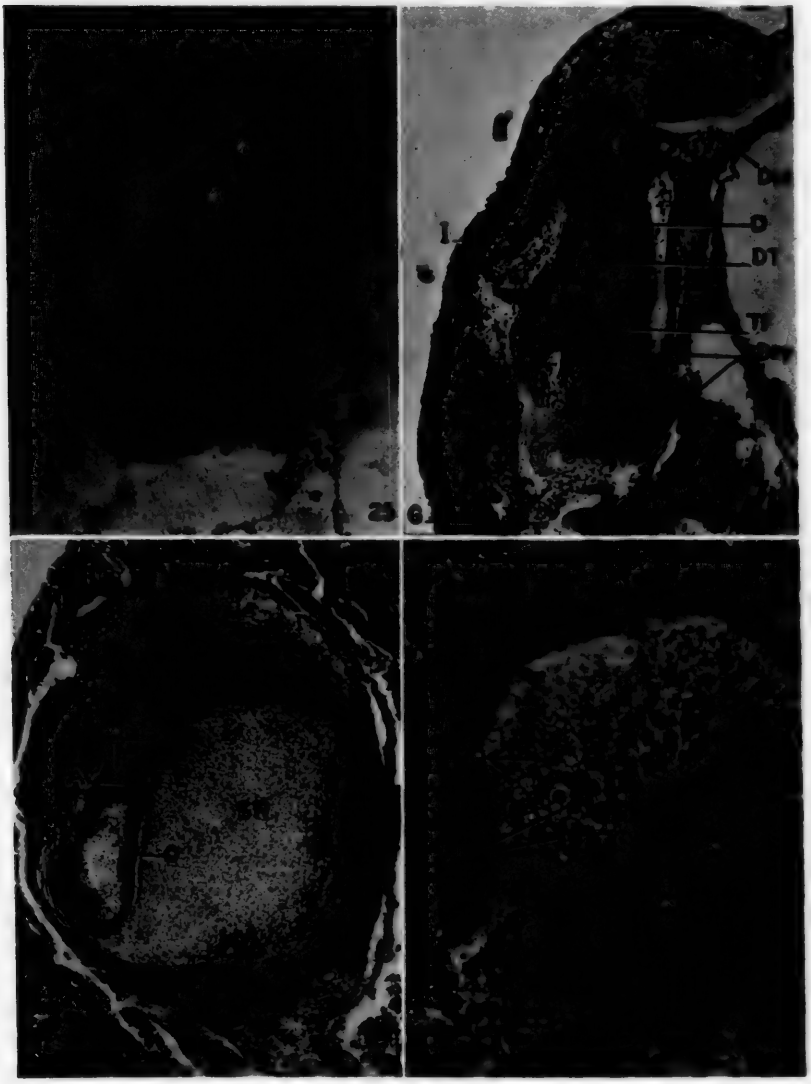

Fig. 25.—Section of lower deciduous canine from the 4 month embryo. The tooth germ from the opposite side of the mandible was transplanted. Fig. 23 shows the results of transplanting the mate to this tooth germ. Original magnification ×40.

Fig. 26.—Section of a rabbit's eye after bearing a portion of a human tooth germ for 20 days. Material was taken from a deciduous canine follicle of a 5 month fetus. Original magnification ×40.

Fig. 27.—Section of a guinea pig's eye retaining a transplant of a human tooth germ from a 5 month fetus. The animal was sacrificed after 24 days. Original magnification ×53.

Fig. 28.—Higher power of area from Fig. 27. Original magnification at *A* ×500.

Losee[21] reported on a method of growing rat tooth germs in vitro and claimed that whole tooth germs were kept alive for 78 days with growth and differentiation of the tissues although the presentation left some doubts. His observations were contrary to the findings of Glasstone,[13] who failed to obtain growth and differentiation, in vitro, of rat tooth germs under identical conditions that produced growth and differentiation of rabbit tooth germs. Similar findings concerning the behavior of tooth germs of mice were observed in the present instance, as homotransplants of these tissues failed to grow in the anterior chamber of the eye, but grew better in the brain than at other sites to which they were transplanted. This is in accord with the findings of both Greene[14] and Browning,[7] who reported that certain embryonic tissues failed to survive intraocular transfer.

Use of the anterior eye chamber as a growth site for transplanted tooth germs has certain advantages over in vitro cultivation. The biological activity of transplants can be easily followed, subcultivation is unnecessary, there is no desmoplastic reaction with the host and removal is easy. In vitro explants become modified by the mechanical conditions and thus tend to flatten out or grow in circular sheets.[10-12] Rabbit tooth germs cultivated in vitro for 20 days by Glasstone[12] compared favorably with 4 days' growth in situ. This rate of growth was slow and explants failed to survive after 25 days and many were lost when transferred to a fresh medium, which had to be done every 72 hours. It is possible that the anlagen of 20 day rat embryos used by Glasstone[13] were too far advanced for satisfactory results as Grobstein[17] in growing embryonic shields in the anterior chamber, after subcultivating them in vitro, obtained tooth differentiation in mice. The age of tooth germs must be an important factor in the growth of these transplanted tissues, for as Nicholson[22] pointed out, embryonic tissues at an early stage of development survive transplantation best. Rapid growth and differentiation observed in the present study showed enamel and dentin formation after 8 days, in a guinea pig's eye, from the anlage of a 21 day rabbit embryo, for once eye transplants of tooth germs became vascularized, growth and development were usually continuous and rapid until calcified tooth structures were formed.

Growth of transplanted tooth germs was always limited to the anterior chamber where there was not a single case of rupture of the cornea by the transplant. Mechanical restrictions and intraocular pressure evidently had a limiting effect on the potential size of these transplants. Microscopic study showed that the pulp and supporting tissues were more compact and proportionately more dentin and enamel formed in the anterior chamber than at other sites. Cartilage, bone and loose connective tissue increased the bulk of transplants in the abdomen or axilla, but the actual size of recovered tooth structures was about the same as that grown in the eye.

Variations between the growth of heterologous and homologous transplants of tooth germs were not significant and as Greene[14] reported, survival and growth of heterologous transplants of embryonic tissues are attained in the anterior chamber, but not at other bodily sites. Transplants of embryonic tis-

sues have one of three fates—to be resorbed at once, to grow for a while and then resorb, or to grow for indefinite periods.[29] This could account for the fact that animals of the same age, sex, and source, receiving identical material, showed variations in the growth and differentiation of these transplanted tooth germs. Woodruff and Woodruff[31] pointed out that the reason eye transplants of tissues survive better than transplants to other sites, is due to the failure of lymphocytes to accumulate readily in the anterior chamber and that vascularization usually takes place before cellular attack can occur. Lack of initial growth could be due to the absence of a stimulating factor for growth being transmitted along with the explanted tissues to the new growth site. After 3 or more weeks the host usually developed an immune reaction and evidences of regression became apparent in established growths. This was confirmed by the presence of osteoclasts and multinucleated giant cells associated with newly formed dentin, indicating a foreign body reaction. Variations in the vascularity of established grafts, during the terminal days of gestation in pregnant animals, indicated a limited effect on transplants.

Variations in the growth and differentiation of ameloblasts may have been due to several factors. When they undergo certain chemical and histological changes to become functional, ameloblasts lose their capacity to multiply as they become concerned with the formation of enamel.[9, 23, 24, 27] The length of these cells, as Addison and Appleton[1] stated, indicates their functional activity or status, thus those that are to form enamel rods become long and those that are not to become functional remain short. Lack of sufficient fluids or of an adequate blood supply may be responsible for the frequent degeneration of these cells. In the present study differentiation and growth of the internal epithelium of the enamel organ were greater than that reported by Glasstone[12] who did not obtain the actual formation of enamel when cultivating tooth germs in vitro.

It is of particular interest in the present study that ameloblasts either functioned normally or degenerated and did not persist as in an ameloblastoma. Robinson[25] found that enamel formation was always absent in ameloblastomas and Bernier[3] in a later report substantiated this. Cystic degeneration is the end result in an ameloblastoma, but in transplants of tooth germs the internal and external epithelium often degenerate without forming cysts. Some of the epithelial remains, in the present instance, resembled the primitive type of cells described by Thoma[26] in his review of ameloblastomas. On failure to form enamel in the anterior eye chamber, ameloblasts lost their tall columnar structures and formed clusters or cords of epithelial cells, epithelial pearls, keratinized material, and sometimes cysts.

Huggins, McCarrol, and Dahlberg[20] attempted to form teratoma-like structures by transplanting tooth germ epithelium to certain locations in the bodies of dogs. Hahn[18] made similar attempts by transplanting this material directly to the ovaries of bitches and obtained epidermoid inclusion cysts. It was significant to Huggins, McCorrol, and Dahlberg, that this transplanted tissue

failed to form cysts but formed epithelial pearls and large islands of basal cells with squamous cell centers. The results of these investigators were similar to those obtained in the present instance.

When a normal relationship was lost between the ameloblasts and the odontoblasts, independent dentin formation continued in either a recognizable tubular pattern or in islands. This resulted in an amorphous formation of tooth structure. Boyle, Bessey, and Howe,[6] who reported on changes in dentin during scurvy, stated that dentin formed after a disorientation of odontoblasts is often of inferior quality and resembles the secondary dentin—osteodentin, pulp bone, or calcific scar tissue—of human teeth.

Fig. 29.—Section of a guinea pig's eye after bearing a transplant of tooth germ material as seen in Fig. 30 for 25 days. A canine tooth germ of a newborn human being supplied this material. Original magnification ×140.

Fig. 30.—Section shows several bands of tooth germ tissue from the same source as those used in the above case. Original magnification ×150.

The presence of multinucleated giant cells and osteoclasts near or in lacunae along one side of a formation of dentin indicated an immune reaction on the part of the host. As Green[14] has stated, the stroma of transplanted tissues is replaced by a new stroma supplied by the host who supplies the immune reaction. Osteocytes or atrophic included cells were often found in dentin, especially near areas that showed resorptive changes. According to Weinmann and Sicher,[28] osteocytes are essential to the normal function of bone tissue, but they have a limited life span and cannot be regenerated by mitotic division, and when these cells near the end of their life span, they must be removed and replaced with young and vital osteocytes. This could explain the constant revision of osteodentin and why transplants of tooth germs if allowed to remain in the anterior chamber for longer periods had most of the dentin replaced by osteodentin that closely resembles bone. It is of interest that Wol-

bach and Howe,[30] in reporting on disturbances in dentin during scurvy, stated that odontoblasts could transform into osteoblast-like cells. In an additional report on scurvy, Boyle[4] found that odontoblasts were always changing, which indicated that dentin was continually undergoing revision. There is a similarity between Boyle's findings and those in the present study for scar tissue resembling that found in the dentin of guinea pigs suffering from scurvy was found in both instances. This was illustrated when the odontoblasts, as such, had disappeared and irregular shaped cells with large nuclei were found associated with this amorphous osteodentin. The "tumor formations," reported by Burns, Orten, and Smith[8] in their study of Vitamin A deficient rats appear very much like the islands of osteodentin that were frequently found in the present experiments, and this raises a question concerning the extent of the effect of embryonic pulp tissues on adjacent mesenchymal tissues.

Ameloblasts were observed during their different morphologic stages in this study and it appears that the essential formation of each enamel rod is dependent upon the function of a single ameloblast. Pre-enamel rods were identified as direct extensions of single ameloblasts. This is not in agreement with Wasserman,[27] who stated that not one ameloblast but several contribute to each enamel prism.

SUMMARY

Whole or parts of tooth germs will survive transplantation from man to lower animals and between lower animals to the anterior chamber of the eye. The exception to this was found in homotransplants of the anlagen of 14 or 15 day tooth germs in mice, but these tissues survived transplantation to other sites. The morphology of the developing tooth was lost when the internal epithelial layer, the ameloblasts, failed to survive. When ameloblasts and odontoblasts were separated from each other dentin usually formed and occasionally enamel was formed. Degeneration of ameloblasts resulted in the formation of cords or clusters of epithelial cells with pearl formation and with occasional appearance of cysts. Transplants of long standing underwent revision and calcified tooth structures were gradually replaced by an osteoid tissue that closely resembled bone. Growth of these transplants never exceeded the anterior chamber. Transplants of human tooth germs to guinea pigs' eyes and transplants of rabbit tooth germs to rabbits' eyes gave the best results.

This study was made possible through the aid and cooperation of several interested people and organizations. The author is particularly indebted to the following: Dr. Paul E. Boyle, Professor of Oral Histology and Pathology at the Graduate School of Medicine of the University of Pennsylvania; Dr. H. S. N. Greene, Brady Professor of Pathology at Yale University School of Medicine; Abraham Spanel; the United States Public Health Service; the Connecticut graduate chapter of the Delta Sigma Delta dental fraternity; and Dr. Morton J. Loeb.

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PRELIMINARY STUDIES ON THE PRESENCE OF ACID PHOSPHATASE IN CERTAIN ORAL MICROORGANISMS

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THE presence of enzymes in whole saliva capable of releasing phosphate from organo-phosphate compounds has been repeatedly demonstrated following the report of Demuth.[1] Since that time there has been constant speculation as to the origin of the phosphatases in saliva. The most generally accepted view is that saliva per se contains little or no phosphatase activity, but rather that it originates in the bacterial and cellular debris present in native saliva. Eggers-Lura[2] and Dentay and Rae[3] have reviewed the earlier literature on the subject of saliva phosphatases.

Since some preliminary experiments in our laboratory using salivas which had been passed through bacteriologic filters or collected directly from Stensen's duct, were in conformity with this view, a study was undertaken to assess the phosphatase activity of some of the microorganisms usually present in saliva. The present report is concerned with the results of a survey of a number of aciduric oral bacteria for the ability to hydrolyze a monophosphate ester within the acid pH range.

EXPERIMENTAL

The lactobacilli, micrococci, aerobacter species, and some of the streptococci employed in this study were originally isolated from pH 5.0 tomato juice agar spread-plates of salivas of children and adults. Other strains of streptococci were isolated from crystal violet-azide-blood plates of saliva. The latter medium had the following composition per liter:

Brain-heart infusion broth (Difco)	37.0	grams
Crystal Violet	0.002	grams
Sodium Azide	0.1	grams
Agar	20.0	grams
Distilled Water	to 1	titer

Defibrinated sheep blood was added to a final concentration of 5 per cent just prior to pouring plates.

After the various strains had been purified by replating they were kept on tomato juice agar or brain-heart infusion agar stabs to which a pinch of calcium carbonate had been added. For phosphatase tests the organisms were grown at 37° C. for 24 to 48 hours in the tomato juice-tween broth described by Rogosa and Mitchell.[4] The bacterial cells were then harvested by centrifuging, washed twice in distilled water, and resuspended in sufficient distilled water to give a bacterial suspension 10 per cent by volume. One ml. of this suspension was used per test.

Received for publication Nov. 9, 1951.

The substrate used for the phosphatase tests was disodium p-nitrophenyl phosphate (Sigma); 0.2 ml. of a 0.2 per cent aqueous solution being used for each test. On hydrolysis by acid phosphatase the substrate which is initially colorless gives rise to p-nitrophenol and phosphate. The p-nitrophenol is colorless at acid reactions but p-nitrophenolate, formed at alkaline pH reactions, has an intense yellow color. Prior to use the substrate was purified of free p-nitrophenolate by two extractions with butyl alcohol and one extraction with ether as recommended by Bessey, Lowry, and Brock.[5] Stock solutions thus purified were stable for at least one week if kept frozen.

The tests with pure cultures of bacteria were performed as follows:

1. Add 1 ml. of a 10 per cent bacterial suspension to 2.0 ml. of an appropriate buffer solution in a Wasserman tube.
2. Add 0.2 ml. of a 0.2 per cent solution of disodium p-nitrophenyl phosphate and mix the contents.
3. Incubate at 37° C. for one hour.
4. Add 0.1 ml. of 10 per cent NaOH.
5. The test is read visually as 0, ± +, +, or +++ depending on the presence and intensity of the yellow color.

The sodium hydroxide had the dual function of stopping the reaction and permitting development of the yellow color of the p-nitrophenolate. Gross variations in intensity were easily determined by visual inspection. When quantitative results were desired the suspensions were cleared by centrifugation and the color intensity of the supernatant was read in the Bausch and Lomb Monochromatic colorimeter at 430 millimicrons. Appropriate blanks were run with each group of tests. In addition each individual test was made to serve as its own blank by acidifying with concentrated HCl diluted 1 to 10 which changed the yellow p-nitrophenolate to colorless p-nitrophenol. The resulting density reading subtracted from the initial density reading gave the corrected density which is proportional to the degree of the hydrolysis of the substrate and thus a measure of phosphatase activity. Under these conditions reagent blanks are negligible if the substrate is carefully freed of p-nitrophenolate before use.

RESULTS

Paraffin stimulated salivas collected from a group of 30 adults ranging from 20 to 35 years in age were examined for phosphatase activity as follows. After thorough mixing of the specimen on a shaking machine, 0.2 ml. of undiluted saliva was added to 2.5 ml. m/30 phosphate of pH 6.0 and 0.2 ml. of the phosphatase reagent solution. Tubes were incubated one hour at 37° C.

In addition tubes of Snyder test medium[6] were inoculated with 0.2 ml. of undiluted saliva. One tenth ml. of a 1 to 10 dilution of saliva in 0.05 per cent yeast extract solution was used for duplicate pour plates of the specific lactobacillus medium of Rogosa[7] and also spread on the surface of duplicate plates of pH 5.0 tomato juice agar for lactobacillus counts. The results of these tests are summarized in Table I in which the degree of phosphatase activity of each saliva sample is compared with the results of the Snyder test and the total

lactobacillus count. Inspection of this table indicates a general trend toward agreement between the three tests. Two notable exceptions are saliva samples Nos. 14 and 15. Tomato juice plates of these salivas contained many colonies of gram negative rods which gave strongly positive reactions for both acid and alkaline phosphatases when tested in pure culture.

TABLE I

A COMPARISON OF THE ACID PHOSPHATASE ACTIVITY OF SALIVA WITH THE SNYDER TEST AND LACTOBACILLUS COUNT

SAMPLE NO.	PHOSPHATASE ACTIVITY	SNYDER TEST*	LACTOBACILLUS COUNT**
12	0	0	0
16	0	0	0
22	0	0	0
26	0	0	0
1	0	+	0
3	0	+	+
5	0	+	+
11	0	+	+
20	0	+	+
28	±	+	+
29	±	++	±
4	±	++	+
17	±	++	++
18	±	++	++
19	±	++	++
23	±	++	++
21	±	+++	+++
24	±	+++	+++
14	+	0	0
15	+	0	0
27	+	++	++
7	+	++	+++
9	+	+++	+++
10	+	+++	+++
30	+	+++	+++
6	++	++	++
13	++	++	+++
2	++	+++	++
25	++	+++	+++
8	+++	+++	+++

*"Caries activity" based on color changes described by Snyder 0 = no activity, + = slight activity, ++ = definite activity, +++ = marked caries activity

**Lactobacillus counts scored as follows: 0 = negative, ± = 1000 per ml., + = 1,000–10,000 per ml., ++ = 10,000–100,000 per ml., +++ = greater than 100,000 per ml.

The next step was to test pure cultures of lactobacilli and other organisms isolated from saliva for phosphatase activity. Approximately two hundred such cultures were screened. It was found that members of the following aciduric groups possessed an acid phosphatase; lactobacilli, streptococci, and micrococci. In addition a number of gram-negative rods resembling *Aerobacter aerogenes* were found to possess pronounced phosphatase activity in both the acid and alkaline pH ranges.

Rogosa, Mitchell, and Fitzgerald[8] have isolated and characterized the lactobacilli found in the salivas of children. A number of these strains were tested for phosphatase activity as shown in Table II, which also shows how the visual estimation of phosphatase activity relates to the colorimetric findings.

TABLE II

ACID PHOSPHATASE ACTIVITY OF ORAL LACTOBACILLI*

STRAIN NO.**	NAME	TYPE	DENSITY***	VISUAL READING
360	L. casei	Homofermentative	0.208	+
530	L. casei	Homofermentative	0.215	+
879A	L. casei	Homofermentative	0.284	+
844A	L. casei	Homofermentative	0.292	+
H101	L. casei	Homofermentative	0.174	+
154	L. acidophilus	Homofermentative	0.155	+
849	L. plantarum	Homofermentative	0.138	±
127	Lactobacillus species	Homofermentative	0.174	+
9	L. brevis	Heterofermentative	0.237	+
175	L. brevis	Heterofermentative	0.398	++
850C	L. brevis	Heterofermentative	0.284	+
11	L. fermenti	Heterofermentative	0.583	++
20	L. fermenti	Heterofermentative	0.569	++
83	Lactobacillus species	Heterofermentative	0.398	++
355	Lactobacillus species	Heterofermentative	0.328	+

*Conditions; m/30 phosphate buffer pH 6.0, incubated 1 hour at 37° C.
**Refers to N.I.D.R. Strain number.
***Refers to corrected density based on transmission at 430 mu; reagent blank set at 100 per cent transmission. Density = 2-log T.

It is apparent that all of the oral lactobacillus strains tested possessed demonstrable phosphatase activity at pH 6.0. Further tests showed that this activity was at a maximum between pH 5.0 and 6.0, decreasing at higher pH values to a minimum at pH 8.0 or above.

Similar tests were performed using a number of strains of streptococci isolated from the saliva. All of the strains were acidogenic, producing a terminal pH of 4.5 or lower in glucose or sucrose broth. On the basis of sugar fermentations, salt tolerance, and colonial morphology on 5 per cent sucrose agar the strains were provisionally separated into three groups, i.e., *Streptococcus mitis*, *Strep. salivarius* and enterococcus. Although the phosphatase activity, when present, is readily apparent in the presence of m/30 phosphate it can be shown, by varying the phosphate concentration or using other buffers

TABLE III

ACID PHOSPHATASE ACTIVITY OF ORAL STREPTOCOCCI*

SALIVARIUS GROUP		MITIS GROUP		ENTEROCOCCUS GROUP	
STRAIN NO. **	DENSITY***	STRAIN NO.	DENSITY	STRAIN NO.	DENSITY
32B	.012	21R	.180	29B	.812
10R	.015	24R	.209		
18R	.562	15B	.263		
3B	.648	13R	.649		
12R	.713	2R	.656		
1R	.781	17B	.671		
20R	.782	4R	.699		
9B	.804	15R	.842		
33B	.845	30B	.845		
17R	.888				
8R	.916				

*Conditions: Maleate buffer 0.1 M, pH 5.2, incubated 1 hour at 37° C.
**Refers to N.I.D.R. strain number.
***Refers to corrected density based on transmission at 430 mu; reagent blank set at 100 per cent transmission.

that the phosphate ion partially inhibits the enzyme. The maleate buffer described by Smith[9] is not inhibitory and proved to be a satisfactory replacement for phosphate buffer in these tests. Table III shows the phosphatase activity of a number of strains of oral streptococci in m/10 maleate buffer at pH 5.2. As in the case of the lactobacilli, most of the members of this group exhibit appreciable acid phosphatase activity.

DISCUSSION

There is evidence in the dental literature to suggest that the phosphatases of saliva may be exogenous in the sense that they are not present in the original salivary gland secretion. It has been our experience, just as that of other investigators, that saliva passed through bacteriological filters or collected directly from the salivary ducts exhibits no phosphatase activity if tested under conditions which preclude the growth of contaminating microorganisms. The evidence in the present paper indicates that at least three groups of microorganisms commonly encountered in saliva exhibit acid phosphatase activity. Some further experiments to be detailed in future publications indicate that this bacterial phosphatase has points of similarity to acid phosphatases described in tissues of higher organisms. For example phosphate inhibits the enzyme to some extent; it is markedly inhibited by fluoride, and apparently does not require Mg^{++} for activity.

Dentay and Rae[3] using a strain of *L. acidophilus* of dairy origin and disodiumphenylphosphate as the substrate were unable to detect phosphatase in this organism. On the assumption that "*L. acidophilus* constitutes an appreciable bulk of the oral flora" these workers concluded that the phosphatase in saliva is "probably derived from cellular debris and food residues with a lesser contribution by microorganisms." This assumption may have to be revised in the light of additional evidence. For example, Bibby, Volker, and Van Kesteren[10] and Stralfors,[11] among others, have demonstrated that the total of all types of lactobacilli amounts to only a very small fraction of the salivary microflora, the ratio of lactobacilli to streptococci being on the order of 1 to 1000. Secondly, Rogosa, Mitchell, and Fitzgerald,[8] have found that strains which can be characterized as *L. acidophilus* occurred with a frequency of about 10 per cent in a group of over 400 strains of lactobacilli isolated from tomato juice agar plates of salivas. Finally, reference to Table II in this paper indicates that the strain of *L. acidophilus* of salivary origin is relatively weak in phosphatase activity when compared with other salivary lactobacilli. In view of the fact that most of the streptococci tested were relatively active in respect to acid phosphatase and that these organisms are numerically predominant in the oral microflora it would seem not unreasonable to suggest that these and other microorganisms may contribute to a very considerable degree to the acid phosphatase activity of saliva. Any decision as to the precise extent of this contribution obviously must await an accurate evalution of the role of other exogenous salivary constituents such as cellular debris or food residues.

The results shown in Table I, while based on only 30 cases, suggest that a parallelism may exist between acid phosphatase activity, Snyder test activity,

and the lactobacillus count of saliva. Since both of the latter tests are used as measures of caries activity the feasibility of using the phosphatase test as a supplemental procedure for this purpose is now being explored. Such a test, if applicable, would have the advantage of being extremely simple to perform since no special equipment is needed, results may be read visually after one hour, and it would readily lend itself to mass survey techniques.

SUMMARY

The acid phosphatase activity of whole saliva and of some of the microorganisms present in saliva has been studied using sodium p-nitrophenyl phosphate as the substrate. Members of the following groups have been found to possess an acid phosphatase; lactobacilli, streptococci, micrococci, and aerobacter.

In an initial trial with 30 cases, generally parallel results were obtained between acid phosphatase activity, Snyder test activity, and lactobacillus counts of saliva suggesting further study of the acid phosphatase activity of saliva as an additional laboratory test for caries activity.

The assistance of Mr. William L. Poole in many of these experiments is gratefully acknowledged.

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DENTAL CARIES AND GINGIVITIS IN MALES 17 TO 20 YEARS OLD (AT THE GREAT LAKES NAVAL TRAINING CENTER)

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A LARGE number of enlistees from various parts of the United States were examined at the Great Lakes Naval Training Center. It was felt that the analysis of dental disease in this group would provide a good cross sectional sampling of young adult American men. These young men came predominantly from the Central, Northeastern, and Southeastern states with a few from scattered areas west of the Mississippi River. All enlistees were males between the ages of 17 and 20 years and all were white.

METHODS AND MATERIALS

Naval enlistees entering the Great Lakes Naval Training Center during 1949 to 1950 were examined for dental decay and gingivitis without selection. All examinations were made by the same examiners—two for caries and one for gingivitis. The teeth and gingivae were examined using only a mouth mirror and explorer under excellent lighting conditions.

Caries.—The caries rate was determined according to the method outlined by Klein, Palmer, and Knutson.[2] Results were tabulated as the number of DMF (Decayed, Missing, Filled) teeth and tooth surfaces.

Gingivitis.—Gingivitis was assessed according to the method outlined by Schour and Massler;[7] Massler, Schour and Chopra;[6] and Massler and Savara.[5] The results were tabulated as the number of papillary (P), marginal (M), and attached (A) gingival areas affected in each mouth. For statistical convenience, the results were also tabulated as the sum of P+M+A.

FINDINGS

Dental Caries Experience.—Table I and Fig. 1 show that the dental caries experience in this sample is fairly close to the averages previously reported by Klein and Palmer[3] and by Hollander and Dunning.[1] The distribution of different degrees of caries is shown in Fig. 2.

The opinions and assertions of the authors are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

This paper is based on a joint investigation by the University of Illinois College of Dentistry and the Dental Department, Administrative Command, United States Naval Training Center, Great Lakes, Ill., with the approval and support of the Bureau of Medicine and Surgery, Navy Department.

Research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service, supported the phase of the study conducted by the University of Illinois College of Dentistry.

Received for publication, Nov. 11, 1951.

TABLE I

DENTAL CARIES EXPERIENCE (DMF) AND GINGIVITIS (PMA) IN MALES 17 TO 20 YEARS OF AGE AT THE GREAT LAKES NAVAL TRAINING CENTER

AGE IN YEARS	NUMBER EXAMINED	AVERAGE NUMBER DMF* TEETH OR SURFACES PER PERSON*		% PERSONS AFFECTED BY GINGIVITIS	AVERAGE NUMBER GINGIVAL UNITS AFFECTED PER PERSON**				CORRELATION (r) BETWEEN DMF SCORE AND P+M+A SCORE
		TEETH	SURFACES		P	M	A	P+M+A	
17	1515	9.8	21.3	65	4.0	1.1	0.0	5.1	0.098
18	1485	12.0	27.2	66	4.0	1.1	0.0	5.1	0.17
19	696	11.7	25.1	66	4.1	1.3	0.0	5.4	0.13
20	347	12.5	27.8	68	4.6	1.2	0.0	5.8	0.127
17-20	4043	11.3	25.2	66	4.05	1.15	0.0	5.2	0.127

*D = number of decayed teeth or tooth surfaces
M = number of missing teeth or tooth surfaces
F = number of filled teeth or tooth surfaces

**P = number of gingival papillae affected
M = number of gingival margins affected
A = number of attached gingival areas affected

TABLE II. DISTRIBUTION OF GINGIVITIS IN MALES 17 TO 20 YEARS OF AGE AT THE GREAT LAKES NAVAL TRAINING CENTER

AGE (IN YEARS)	NUMBER EXAMINED	NO GINGIVITIS	SOME GINGIVITIS	DEGREE OF GINGIVITIS* (IN PERCENTAGE OF PERSONS AFFECTED)				
				VERY MILD	MILD	MODERATE	SEVERE	VERY SEVERE
17	1515	35.3%	64.7%	13.9%	14.9%	17.0%	9.8%	9.1%
18	1485	33.9	66.1	18.4	17.9	13.7	7.6	8.6
19	696	34.2	65.8	16.1	16.8	15.1	8.2	9.6
20	347	32.3	67.7	20.2	13.5	17.6	8.4	8.1
17-20	4043	34.3	65.7	16.5	16.2	15.5	8.6	8.9

*Very Mild = ½ Average or less (P+M+A; 1-2)
Mild = ½ to 1x Average (P+M+A; 3-5)
Moderate = 1x to 2x Average (P+M+A; 6-10)
Severe = 2x to 3x Average (P+M+A; 11-15)
Very Severe = More than 3x Average (P+M+A; 16+)

TABLE III. AVERAGE CARIES EXPERIENCE (DMF TEETH AND TOOTH SURFACES) IN PERSONS WITH DIFFERENT DEGREES OF GINGIVITIS

DEGREE OF GINGIVITIS*	NUMBER IN EACH CATEGORY	AVERAGE NUMBER DMF TEETH OR TOOTH SURFACES PER PERSON	
		TEETH	SURFACES
None	1387	11.01	24.27
Very Mild	666	11.56	26.10
Mild	655	12.25	27.67
Moderate	627	11.54	25.64
Severe	347	11.18	25.09
Very Severe	361	10.66	22.78
All	4043	11.3	25.2

*See Table II for more complete explanation.

Gingivitis Experience.—Table I and Fig. 1 show the average number of gingival units affected at each age level. The findings in this group compare very closely with the findings by Massler, Cohen, and Schour.[4] The frequency distribution of different degrees of gingivitis is shown in Table II. Fig. 3 indicates that the distribution of different degrees of gingivitis is hyperbolic in contrast to the "normal" distribution of caries as shown in Fig. 2.

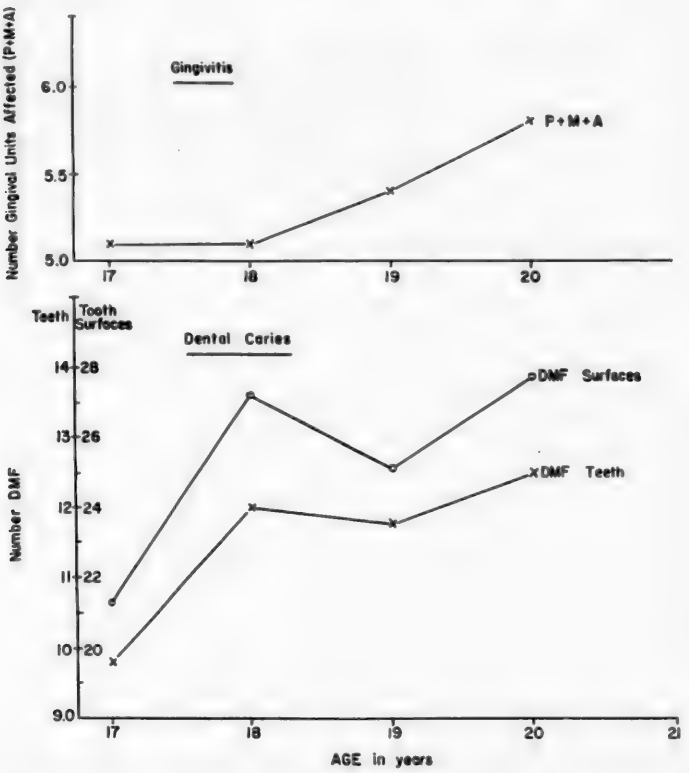

Fig. 1.—Incidence of dental caries (number of decayed [D], missing [M] and filled [F] teeth and tooth surfaces per person) and of gingivitis (number of papillary [P], marginal [M] and attached [A] gingival units affected per person) in 4,043 males, 17 to 20 years of age.

The Relation of Caries Experience and Gingivitis.—The relation between periodontal disease and dental caries has always been an intriguing clinical question. Clinical opinion holds that caries and periodontal disease are mutually exclusive. However, this does not mean that the same is also true in the relation between caries and gingivitis. Massler and Savara[5] investigated the correlation between caries experience and gingivitis in teen-age children (aged 14 to 18 years) and discovered neither a positive nor negative relationship. The relationship was tested by (a) computing the coefficient of correlation between the average DMF score and P+M+A score (as in Table I), and (b) by computing the average DMF score in groups with none, mild, moderate and severe gingivitis

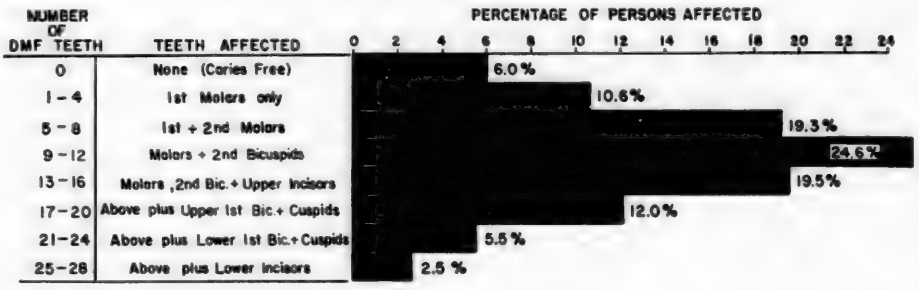
Distribution of Dental Caries (DMF Teeth)
in Males 17 – 20 Years of Age
NUMBER OF DMF TEETH
TEETH AFFECTED
PERCENTAGE OF PERSONS AFFECTED
0 2 4 6 8 10 12 14 16 18 20 22 24
0 None (Caries Free) 6.0%
1 – 4 1st Molars only 10.6%
5 – 8 1st + 2nd Molars 19.3%
9 – 12 Molars + 2nd Bicuspids 24.6%
13 – 16 Molars ,2nd Bic.+ Upper Incisors 19.5%
17 – 20 Above plus Upper 1st Bic.+ Cuspids 12.0%
21 – 24 Above plus Lower 1st Bic.+ Cuspids 5.5%
25 – 28 Above plus Lower Incisors 2.5%

Fig. 2.—Distribution of dental caries (DMF teeth) in 4,043 males, 17 to 20 years of age, examined at the Great Lakes Naval Training Center.

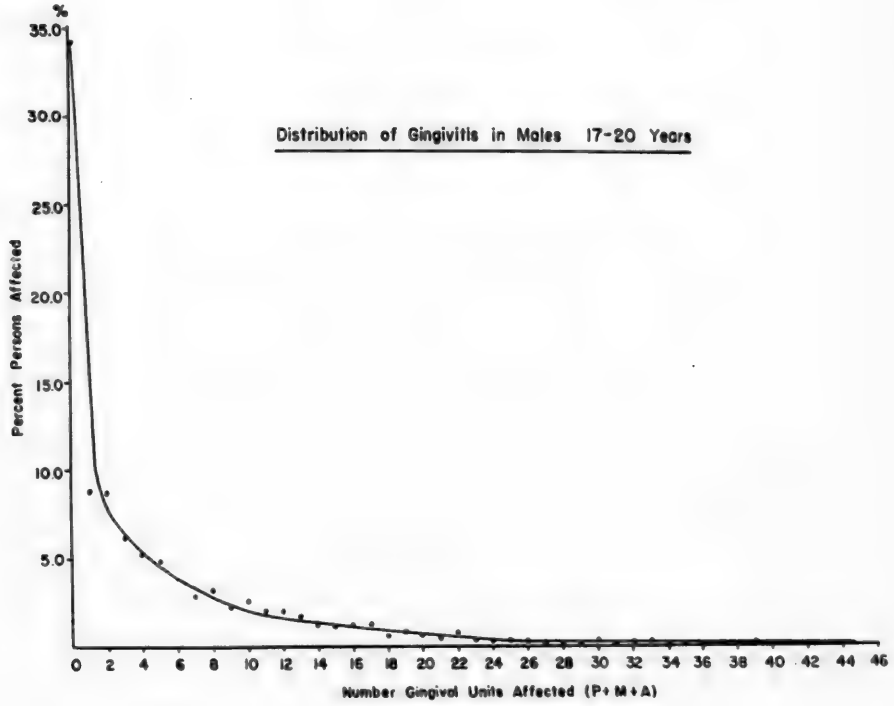

Fig. 3.—Distribution of gingivitis (P+M+A) in 4,043 males, 17 to 20 years of age.

(as in Table III). A similar analysis in this large group of males, 17 to 20 years of age, showed that no relationship existed between dental caries experience and gingivitis (Tables I and III).

DISCUSSION

The opportunity presented itself to study the prevalence of dental caries and gingivitis in a fairly large cross sectional sample of the young adult male population of the United States entering the Great Lakes Naval Training Center. The findings substantiate previous reports on the average caries attack rates in the United States. The present group represented an adequate sample of young adult males living east of the Mississippi River and coming from rural, village, city, and metropolitan areas. We feel therefore that these data represent an accurate cross section of dental caries and gingivitis experience in the young adult American male.

SUMMARY AND CONCLUSIONS

Examination of 4,043 white male naval inductees, 17 to 20 years of age, from various areas in the United States showed them to experience an average of 11.3 DMF teeth and 25.2 DMF tooth surfaces per person and an average of 5.2 P+M+A gingival units affected per person. No correlation either positive or negative could be demonstrated between gingivitis and dental caries experience in this sample of young adult males. This does not negate the necessity for further study of the relationship between caries and periodontal disease in persons in older age groups.

We wish to express our appreciation to Captain C. W. Schantz, DC USN, The Dental Office, for his aid and cooperation; to Dr. Jack Zwemer, who supervised the statistical tabulations by means of I.B.M. cards; to LCDR E. T. Guay, DC USN, for the caries examinations and to W. R. Martin, DN USN, for his active participation in the survey of the enlistees.

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RELATION OF FUNCTION TO CARIES RATE

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THIS report is concerned with an experimental attempt to increase the dental function of a controlled population and describes the effect on the caries rate. It was prompted by observations on various dietaries and their relation to caries incidence which suggested that a high degree of function and caries immunity are commonly associated.[1]

The amount of chewing in length of time or in the number of chewing motions was considered of minor importance compared with the force exerted during mastication. In preliminary tests[2] it was found that raw fruits and vegetables, toast, biscuits, and commercially available chewing gum were not suited for this experiment; foods that were considered to require sufficient vigorous mastication were certain types of bread and sugar cane. Of these two, hard bread seemed to have little appeal to adolescents and their attitude to the chewing of bread would defeat the progress of an experiment for any prolonged period. We therefore finally selected sugar cane, certain varieties of which have considerable taste appeal to youth and at the same time offer a maximum of hard bulk which, during mastication, does not lose its fibrous structure.

King[3] used sugar cane in therapeutic experiments, studying the influence of cane chewing on periodontal structures. The teeth of the population in cane growing areas were reputedly little attacked by caries. An investigation by King and Martin[4] in the West Indies did not entirely confirm this notion, though they stated that cane chewing is a "fast dying habit."

The present study was conducted at Children's Village, Dobbs Ferry, New York. This institution has a population of over 400 boys, fourteen to fifteen years old, with a residence averaging two years. The boys are generally in good health and under constant medical supervision. All the children receive the same standard diet from one central kitchen and the food is distributed before each meal to the various individual cottages in which the boys are housed.

PROCEDURE OF THE EXPERIMENT

Suitable varieties of sugar cane were secured from Florida, Louisiana, and Puerto Rico and were transported and stored under refrigeration at a temperature from 36° to 38° F.*

The ends of the cane sticks were waxed to prevent or delay fermentation and desiccation. Under these conditions little of the cane deteriorated and it could be stored from an average of one month to a maximum of 3 to 4 months.

Five cottages were selected for the daily distribution of one piece of sugar cane per child and vigorous mastication on the part of the children was invited and supervised by house parents. In five other cottages, which served as controls, the children received daily the juice extracted from a fresh piece of sugar cane, the same in size as the pieces handed to the chewing children. The pre-

Received for publication Sept. 20, 1951. Revised by authors Dec. 7, 1951.

*We acknowledge assistance from the Sugar Research Foundation, Inc., New York, N. Y., and technical advice in procurement, selection and storage of the sugar cane.

caution of this control was taken in order to limit the difference between the chewing and the nonchewing groups to the physical structure and chewing requirements of the cane. It served to obviate the necessity of a detailed study of vitamin and mineral content of the particular types of cane used.

Children in the third group of five cottages served as another type of control by receiving neither cane nor juice. This control group provided the normal caries attack rate in the institution.

In order to secure the necessary population, new admissions were accepted to provide at least 100 children in both experimental and control groups. The period of observation was eighteen months for all cases included in the study.

The examinations were carried out by a dentist and a hygienist who charted their findings without knowledge to which group the children belonged. All children had their teeth examined radiographically and clinically at the start and termination of the study. Clinical examination was carried out with mouth mirror and explorer and caries was identified according to the usually accepted standards. In full-mouth bite-wing radiographs caries was identified by any decalcifications penetrating to the dentino-enamel junction.

The cottages that were designated for the cane-chewing groups received daily a bag containing suitably cut pieces of sugar cane of about 6 inches in length and sufficient in number so that each boy would receive daily one piece of cane. This cane was stripped of its hard barklike peel with the aid of knives. Boys with good incisors were advised that they could use their front teeth to peel the cane. After this preparation, the pieces were chewed, whereby emphasis was put on exerting vigor in mastication. Sucking of cane was forbidden by the house parents who supervised the chewing. The time at which the cane was eaten varied in different cottages; usually the rest period after lunch was assigned to the purpose.

The other group of cottages receiving the syrup were supplied daily with freshly milled juice. The amount distributed equaled closely the amount of cane juice that the boys in the chewing group received by extracting it themselves by mastication. The syrup was given either plain or mixed with other foods or liquids when the sweetness was objected to. Most children, though, liked the taste of the juice and no gastric or other disorders were reported from ingestion of the comparatively small amount of juice daily.

The various difficulties encountered during the experiment centered mostly around the problem of obtaining a steady supply of palatable cane. There were several periods during which the cane distributed had no good chewing qualities, or was poor in taste, and therefore was rejected or at least not readily accepted by the boys.

A few children did not care for the cane; these were mostly children who had initially poor teeth. Yet, as all children remained in the two control groups, we did not exclude them in the counting of the caries experience in the chewing group either.

Some children in the control groups made every effort to secure sugar cane for its taste and flavor. They attempted to circumvent the regulations estab-

lished by the administration. No record of their success was possible; every effort was made to keep these irregularities at a minimum.

The number of children observed and examined in the experiment exceeds seven hundred. As the children enter the village at any time throughout the year many who initially entered the experiment were discharged after periods of observation of less than eighteen months. A great number of children admitted later to the experiment did not stay sufficiently long. Furthermore, all those who entered the experiment during the last eighteen months were, like the other two groups, not included for the final tabulation of the results.

In order to be able to eliminate from the calculations all children who were in the experiment for a period of less than eighteen months, the time of the experiment had to be extended for a period of three years from December, 1947, to January, 1951.

In the evaluation of the findings we employed the estimation of the DMF (decayed, missing, filled teeth) rate. The results are summarized in the tables.

TABLE I

	NO. OF CHILDREN IN EXPERIMENT
Cane-Chewing Group (Group 1)	102
Control Groups (Groups 2 and 3)	164
Group Receiving Cane Juice (Group 2)	83
Group Receiving Neither Cane Nor Juice (Group 3)	81

TABLE II

COMPARISON OF DMF TEETH FOR EXPERIMENTAL AND CONTROL GROUPS

	GROUP 1 (CANE CHEWING)	GROUP 2 (CANE JUICE)	GROUP 3 (NO CANE)
Total DMF teeth at initial examination	1173	939	849
Initial DMF average per child	11.5	11.3	10.5
Total DMF teeth after 18 months	1289	1118	1016
DMF average per child after 18 months	12.6	13.5	12.6
Total DMF increase	116	179	167
Mean DMF increase per child	1.1	2.2	2.1
Standard deviation	1.4	1.9	2.1
Standard error of mean	0.14	0.21	0.23

The mean DMF increase per child for both the control group and the group receiving cane juice is about twice as large as the mean DMF increase per child in the group receiving whole cane. In a statistical analysis the data were tabulated according to the number of cases showing increases in DMF teeth of zero, one, two, three, and so on and the following calculations were made for each group:

$$\text{The mean DMF increase per child. } m = \frac{\Sigma f(x)}{N}$$

$$\text{Standard deviation } \sigma = \sqrt{\frac{\Sigma f (x-m)^2}{N}}$$

$$\text{Standard error of mean, SE} = \frac{\sigma}{\sqrt{N}}$$

whereby x equals the increase in DMF teeth, f the frequency of occurrence of x, and N the number of children in each group.

The significance of the differences between means was tested by calculation of the critical ratio,

$$CR = \frac{m_1 - m_2}{\sqrt{S E_1^2 + S E_2^2}}$$

where m_1 and m_2 refer to the mean DMF increase per child for each group, respectively, and S E_1 and S E_2 refer to the standard error of m_1 and m_2, respectively.

In comparing the cane-chewing group and the juice-drinking group, this ratio has the value of 4.2. Comparing the chewing group with the other controls, the value is 3.1, and 0.3 in comparing the two control groups.

The chances of the difference in DMF increase between the cane-chewing group and either of the other two control groups occurring by chance alone is less than 0.3 per cent. These differences, therefore, may be considered significant. The ratio of 0.3 in comparing the juice-drinking group with the children receiving neither cane nor juice indicates that there is no significant difference between the means of these groups.

DISCUSSION

The difference of about 50 per cent in the occurrence of new teeth affected by caries in the chewing and the nonchewing group can be attributed to the additional function provided by the daily mastication of sugar cane. The actual inhibiting process is open to argument, between a purely mechanical detergent action of the fibrous food, and a metabolic effect of function on the organic structure of the teeth, causing an increased resistance to caries. Another undetermined factor may be at play.

Previously reported findings on rat and monkey teeth suggested that disuse may have an effect on the structure[5, 6] of the teeth in the way it affects other organs with mechanical functions in the body. These studies had no direct reference to caries and the findings are not indicative regarding the particular part of the dental structure affected by disuse.

The results are not too startling compared with caries reduction reported obtainable by other means. Discrepancy of effort would be all the more in favor of, for instance, fluorine as the present results of a 50 per cent caries reduction were obtained under considerable technical difficulty of supply and constant supervision, yet we feel that we may follow a physiological route of caries elimination. The described experiment suffered a considerable handicap in the varying quality of the cane, and vigorous mastication was not sufficiently and regularly maintained.

Another difficulty in the experiment consisted in the generally rather poor dental condition at the outset which interfered in many cases with the ability to chew adequately. Nevertheless, these boys, some of them unable to cope with the cane, were not eliminated from the experimental group. The data thereby remained comparable with the control groups.

It will yet have to be investigated under more favorable conditions of cane supply or a, as yet unknown, fibrous substance with taste appeal for children,

whether or how far the results can be further improved and what the practical limitations and optimal benefits will be.

SUMMARY

In an experiment designed to study the effects of vigorous mastication on caries incidence, over a period of eighteen months, 102 children received a daily supply of fibrous sugar cane which they chewed under supervision, 164 controls were again divided into two groups of which one received daily freshly milled cane juice, and the other neither cane nor juice.

The results obtained showed a 50 per cent reduction in the caries rate in the chewing group. A concept relating susceptibility to caries to metabolic changes in the tooth due to underfunction is set forth.

The major difficulties of the experiment were the obtaining of a steady palatable cane supply, and poor dentitions to start with in many of the children which made many in the chewing group unable to cope properly with the cane. The authors feel that the present results do not therefore necessarily reflect the limitations or optimal benefits obtainable from vigorous function as a physiological approach to induce caries immunity.

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ELECTROMOTIVE FORCES AND ELECTRIC CURRENTS CAUSED BY METALLIC DENTAL FILLINGS

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ELECTRICAL phenomena in oral cavities have been described and discussed in many papers published between 1878 and the present time. Solomon, Reinhard, and Goodale[1] abstracted the 24 most important papers published before June, 1933.

The papers up to late 1933 describe different cases and suggest explanations of the observed symptoms. Cases are described in which certain pathologic conditions in the oral cavity were relieved when the metallic restorations were changed so that all were made of one kind of metal, or were completely removed.

Lain and Caughron[2] reported their use of a Weston Model No. 440 microammeter having a range of 75 microamperes, and stated that in only a few cases did they require an instrument having a greater range. They also state correctly that the potential difference of the two restorations could be calculated by taking the product of the observed current and the resistance of the microammeter. They state neither the resistance of the meter nor the magnitude of a potential difference. The chief concerns of the paper are case histories and general observations.

Solomon, Reinhard, and Goltz[3] reported the results of laboratory experiments in which they used saliva and six different restoration metals. They suggest that a current would exist in a normal oral cavity provided an external circuit exists and that soft tissue might supply the external circuit. They state further that it is "obviously impossible" to measure such a current in vivo and then suggest an experiment in vitro, which they performed. This experiment consisted of immersing a gold and an amalgam electrode in saliva. When a microammeter was connected to the electrodes it indicated a current. When both a copper wire and the ammeter were connected in parallel to the two electrodes, the meter indicated "no current." They state that the copper wire simulates the soft tissue external circuit and that therefore "this may be considered evidence that no external circuit exists in the soft tissues of the mouth." This conclusion appears to be quite incorrect. The resistance of the copper wire undoubtedly was very low compared to that of the ammeter and therefore the current through the meter was too small to cause the meter to deflect appreciably. Further, if the copper wire had had a resistance of thousands of ohms, as is the case for the resistance between a pair of dental restorations

The data on which this report is based were part of Naval Research Project NR-182-510. This contract was negotiated by the University of Oklahoma Research Institute.

Received for publication, Nov. 29, 1951.

through the soft tissues, then the meter would have recorded a large deflection. It is clear that these workers were not aware of the fact that the bone fluid* in the tooth and jaw also supplies a very good external circuit.

Solomon, Reinhard, and Goltz then observed the decrease in current with time when the microammeter alone was connected to the two electrodes. From these experiments they concluded that, although an e.m.f. exists between metallic restorations in the mouth, "no current is normally flowing." They suggested that polarization and oxidation of the restorations together with film formation prevent the flow of current. It is evident that their experimental results do *not* lead to this conclusion which rests on the copper wire data. Since the copper wire simulated the soft tissue and since a relatively large current did go through the wire, that current also went through the saliva and the bone fluid between the two electrodes. Thus it appears that their conclusion should have been that a current *is* normally flowing.

Lain, Schriever, and Caughron[4] described experiments which demonstrated the electrical conditions that exist in the case of two fillings made of different metals. It was shown that the bone fluid acting on the two fillings produces an e.m.f. which is nearly as great as that produced by the saliva, each of them being from amalgam to gold through the respective electrolytes. It was shown also how these electromotive forces might be measured and that the two of them would combine in parallel to furnish a current through a microammeter connecting the two restorations. It was pointed out why the current through such a meter would be much greater than that which would exist in the oral cavity under normal conditions.

Reed and Willman[5] described some experiments with pieces of platinum, gold, copper, and amalgam immersed in several solutions including the saliva in an oral cavity. They also connected a large amalgam filling and a gold inlay in an oral cavity, with a gold wire. Hours later they connected the galvanometer to these restorations and then broke the gold-wire connection. The current was then observed to be one microampere. They concluded from these experiments that under truly average conditions in the mouth appreciable currents do not exist. They also stated that currents from 1 to 100 microamperes may exist if a gold and an amalgam filling come in contact. They considered currents of the order of one microampere not to be harmful. It appears that Reed and Willman neither measured the magnitude of an electric current in a normal oral cavity nor measured quantities from which such a current could be calculated. They demonstrated that polarization effects build up relatively rapidly when the fillings are connected with a wire (ammeter), and that these tend to reduce the current.

Reed and Willman also referred to a paper by Mills[6] who found in a statistical survey of 1,000 patients that the incidence of irritation of the oral mucosa was essentially the same for patients with dissimilar metal restorations as for patients having restorations of only one metal or no restorations at all.

*In this paper we shall define "bone fluid" to mean all those electrolytes in the dentin, bone, and tissue through which the electric current passes in going from the bottom of one filling through the tooth and jaw, then through the other tooth to the bottom of the second filling.

Venable, Stuck, and Beach[7] described experiments in which they placed screws, made of various pure metals and alloys, in the fractured radii of dogs, two screws being placed near each other in each radius. Their work convinced them that galvanic action did occur and that the metals were transported as would be expected from their positions in the electromotive series.

MacGreagor and Fickling[8] described three cases in which electrolytic action between Cu-Ag dental splints and Cr-plated steel pins caused ulcerations, bone necrosis, and delayed healing of mandibular fractures treated by bone grafting and extraoral fixation. The grafts survived but sequestra developed from one-half to one inch around the pins. Ulceration, considered to be due to electrolysis, occurred around the screws, projecting points, and soldered connections in the intraoral splint. A recording galvanometer indicated potential differences of 40 to 50 mv, with surges up to 200 mv, between a pin and a splint while in position in the patient. In one case where there existed some inflammation but no ulceration the potential differences were 5 to 10 mv. In seven additional cases treated by using acrylic resin junctions and stainless steel pins no ulceration or necrosis occurred.

Since 1933 several papers have appeared in which are described cases of stomatitis, leukoplakia and oral cancer which were believed to have been caused by electrogalvanic action of dental restorations. One must admit that much of the evidence presented hardly justifies the statement that electrolytic action of dental restorations was the undoubted cause of the pathological conditions.

Aasgaard* reported the potential differences of pairs of restorations of various kinds measured in 21 oral cavities with a vacuum-tube voltmeter of special design. Data on some 170 pairs were given. The largest, 950 mv, was between an aluminum splint and a gold crown; the next largest, 770 mv, was between two amalgam restorations. The average of the potential differences was approximately 135 mv. It can be inferred from his report that he used platinum-iridium tipped probes to contact the restorations and that the restorations were wet with saliva as they are under normal oral conditions.

Aasgaard also reported his experience with a patient who had a four-piece gold bridge and two large amalgam fillings with a potential difference of 330 mv between the bridge and one amalgam filling. Symptoms, such as irritation of the gingiva and the tongue, existed and the patient complained of pain in the tongue and in the palate. When the two amalgam fillings were replaced by baked porcelain the pain in the palate disappeared and the pain in the tongue diminished. The gold bridge was removed and an amalgam filling was found under a gold crown. When this amalgam restoration was replaced with gold the pain disappeared, the tongue regained its normal appearance, and the patient has remained without symptoms ever since. The potential difference between two gold restorations was around 70 mv. This report indicates that the galvanic electrical effects due to dissimilar metallic dental restorations may cause pathologic conditions in the mouth.

*Private communication to Dr. E. S. Lain of Oklahoma City from G. Aasgaard, Bergen, Norway, March 9, 1939.

Venable and Stuck[9] reviewed the literature concerning the use of metals in the fixation of fractures, in the ligation of vessels, and in the support of soft tissues. They state that, "brass, carbon steel, nickel-plated steel, and many other metals and alloys have been tried, but in each case irritation of the tissues, delayed healing, swelling, and sinus formation have resulted. These phenomena, formerly attributed to infection, pressure necrosis, or foreign-body reaction, are now known to be due to intense electrolytic reactions caused by the effects of the body fluids on the dissimilar metals contained in the alloy. . . . Steel appliances plated with nickel, silver or copper usually caused the most severe reactions of all. The plating became cracked or chipped so that two dissimilar metals were side by side in the body fluids. There was much electroactivity in these batteries and tissue damage and bone necrosis inevitably followed . . . So far, we know of only three metals which are sufficiently passive for use in the body, vitallium, tantalum, and 18-8-SMo stainless steel, and of these vitallium is the only one which appears to be uniformly inert."

BASIC CONSIDERATIONS

Several possibilities for sources of electric current exist in an oral cavity containing two or more metallic fillings. First, an electric cell will be formed by two fillings, one gold and one amalgam for example, together with the saliva which is an electrolyte wetting the exposed surfaces of the fillings. Second, these same two fillings also contact the bone fluid through the teeth. Since the bone fluid is also an electrolyte, it, together with the amalgam and gold fillings, constitute another electric cell. Each of these two types of cells has the direction of its e.m.f. from amalgam to gold through the respective electrolyte. A third type of possibility for an electric cell exists. Since a single metallic filling under normal oral conditions contacts saliva at its exposed surface and bone fluid down in the tooth, and since the saliva and the bone fluid are in contact through the tissues, the requirements for a liquid-junction cell are fulfilled. This is illustrated in Fig. 4.

A careful survey of the literature reveals that in no instance has an e.m.f. caused by dental fillings, or the resulting normal current in an oral cavity, been measured, and that in no instance have measurements been reported from which these quantities could have been calculated.

PURPOSE OF THIS INVESTIGATION

The purpose of this investigation was to devise methods, including the necessary theory and apparatus, for determining electromotive forces, electrical resistances, and electric currents associated with metallic dental fillings in place in oral cavities under normal oral conditions. It was also proposed to make such determinations on a large group of persons having normal mouths, and to make measurements in as many pathologic oral cavities as might become available.

EXPERIMENTS

Part I. Pairs of Metallic Fillings.—Attempts to measure the electrical potential differences between pairs of fillings with a standard precision potentiometer having a sensitive galvanometer as a balance indicator, were unsuc-

cessful because duplication of results was not possible. The fillings had such small areas of contact with the saliva and the bone fluid that intolerable polarization effects were caused by the tiny currents required for the galvanometer. It was necessary to devise an apparatus which required the fillings to deliver much less electric charge per measurement.

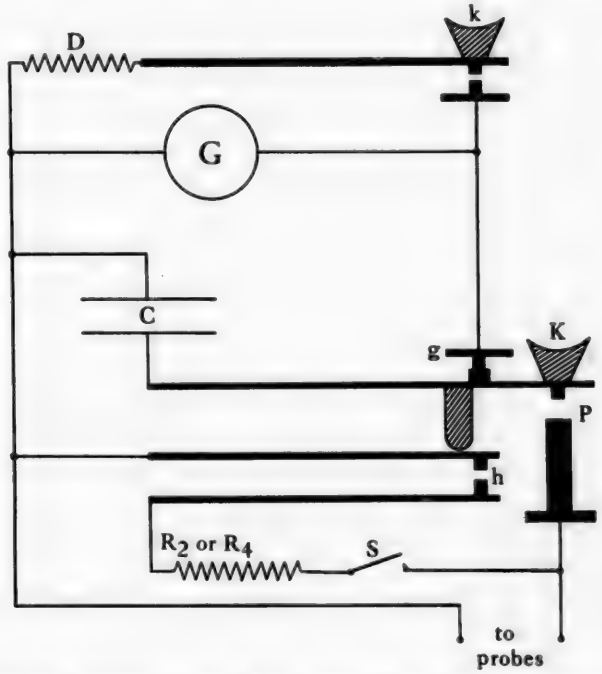

Fig. 1.—Schematic diagram of the electric circuit for the condenser-ballistic-galvanometer potential-difference meter.

k, galvanometer damping key
D, damping resistance
G, ballistic galvanometer
C, a one mf condenser
R_2 or R_4, a resistance which may be connected to probes by closing switch *S*
K, a special triple-contact key which connects R_2 and *C* to probe and, on release, connects *C* to *G*

A schematic diagram of such an apparatus is shown in Fig. 1. This apparatus might be termed a condenser-ballistic-galvanometer potential-difference meter. It does not require a continuous current for its operation, as did the potentiometer galvanometer. *G* is a Leeds & Northrup Type "P" ballistic galvanometer having a sensitivity of 0.004 microcoulomb per mm. deflection on the scale which was 50 cm. from the mirror. While the galvanometer coil was swinging back to the zero position it was damped by closing the auxiliary key (*k*) which connected a critical damping resistance (*D*) across its terminals; this made it possible to bring the galvanometer coil to rest quickly.

The two probes which contacted the two fillings were connected to the terminals marked "to probes." The construction of a platinum-tipped probe is shown in Fig. 2.

When the triple-contact key (K, Fig. 1) was depressed it connected the probe terminals directly across the condenser (C) through contact (P), thus charging the condenser which was a high grade oil-filled condenser having a capacitance of one microfarad. When the key (K) was released to the position shown in Fig. 1, the charged condenser was disconnected from one probe and then connected through contact (g), so that it discharged through the galvanometer.

When it was desired to connect a resistor (R_2), across the probes, the switch (S) was closed. Then when the key (K) was depressed the resistor (R_2) was connected by the closing of the contact (h); this occurred before the condenser was connected by the closing of the contact (P). When the key was released the condenser was isolated before the contact (h) opened; had this not occurred the condenser would have discharged through R_2 instead of through the galvanometer.

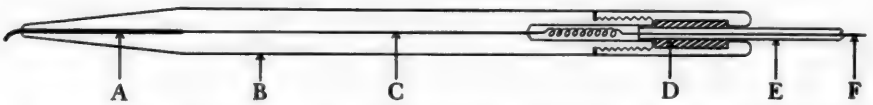

Fig. 2.—Scale drawing of a 7 inch platinum-tipped probe which was used to make electrical contact with a metallic filling.

A, a platinum wire sealed with plastic cement
B, polystyrene rod, 0.5 inch in diameter
C, copper wire sealed with plastic cement
D, a rubber gasket which grips *E*
E, shielded lapel-microphone cable, polyethylene insulation
F, flexible copper conductor within cable

Preliminary measurements indicated that the resistance between a pair of fillings was approximately a half megohm. It requires appreciable time to charge a 1 mf condenser by the e.m.f. generated by two fillings, through a total circuit resistance of ½ megohm. In order to have the condenser at least 99 per cent fully charged, it was necessary to keep the key (K) closed for five seconds. It was convenient to observe the charging time on a sweep second-hand electric clock.

When the resistor (R_2) was connected, the two fillings had to supply current through it as well as to charge the condenser. Since the passage of current through the resistor caused polarization effects at the fillings it was necessary to reduce this current as much as possible. Thus the polarization could be reduced by increasing R_2 and decreasing C. On the other hand, each of these changes decreased the accuracy of the measurements. Experience showed that a good compromise occurred when C was 1 mf and R_2 was 1 megohm. The charging current for the condenser (C) was so small and lasted such a short time that fairly satisfactory reproduction of data was possible.

This condenser-ballistic-galvanometer potential difference measuring apparatus was calibrated by connecting known potential differences, varying from 10 to 1000 mv, to the probe terminals. It was found that the deflection of the ballistic galvanometer was directly proportional to the applied potential difference and that the deflection to the right was the same as the deflection to the left for the same potential difference. The sensitivity of the instrument was found to be 3.3 mv per mm. deflection.

Experimental Procedure.—Students in the School of Medicine, and nurses, both graduate and undergraduate, served as "normal" subjects. Their ages ranged from 18 to 30. A small number of older people also served as subjects. Gold fillings were found to be relatively scarce, especially in the mouths of the younger men and women.

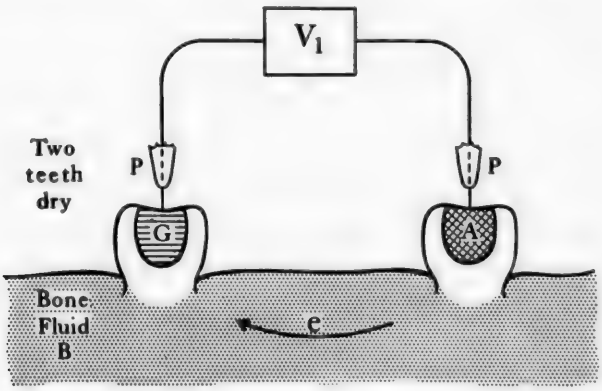

Fig. 3.—Circuit for measuring the e.m.f. caused by the action of the bone fluid on two fillings.

G, a gold alloy restoration
A, a silver amalgam restoration
e, e.m.f. due to contacts of *G* and *A* with the bone fluid
V_1, the potential difference indicated by a meter which does not require a continuous current. Since no current exists, $e = V_1$
P, two platinum-tipped probes which make electrical contacts with the restoration

It was of greatest interest to locate the largest existing electrical effects in each of the mouths studied. Since it was necessary to repeat measurements of potential differences in order to be certain of reproducible results, only a few pairs of fillings could be studied in any one mouth in an hour. Preliminary measurements of V_3, the potential differences between pairs of saliva-wet fillings, were made on various pairs of fillings in an oral cavity, and those pairs were selected which exhibited the greatest potential differences. Some pairs exhibited nearly zero potential difference.

The electrical measurements were carried out with the subject sitting in a reclined position in a barber chair. The two platinum-tipped probes were pressed firmly against the pair of fillings by one research assistant while all the teeth were wet with saliva. The second research assistant pressed down the key (*K*, Fig. 1) for five seconds, released it, and read and recorded the

deflection of the galvanometer. This result was checked by making one or more additional determinations. From this galvanometer reading the potential difference V_3 was later computed.

Next one oral pack (or two if necessary) was put in place, the two teeth were carefully swabbed with 95 per cent ethyl alcohol, and air was blown on both teeth. The potential difference of the dry fillings in the dry teeth was measured as described above. This potential difference is designated V_1 (Fig. 3). The switch (S, Fig. 1) was closed and the potential difference of the two fillings, when connected by a 1 megohm resistor (R_2), was determined. This potential difference is designated V_2. Each measurement was repeated in order to check the work and also to learn whether too great a polarization had occurred; if this did occur then reproducible results were not obtainable.

This same procedure was repeated for one or more additional pairs of fillings in the mouth of this same subject. In some cases it was necessary to swab a pair of teeth several times and to blow air on them from time to time in order to keep them dry. Occasionally it was found impossible to keep the desired teeth sufficiently dry, in which case they were abandoned and another pair was selected.

Other data concerning the subject were also obtained and these together with the three galvanometer deflections were recorded on a report form. This form included blanks for serial number of subject, date, name, age, sex, address, phone number, date and nature of last illness, pH of the subject's saliva, and pertinent information concerning past lesions in the subject's mouth. On a conventional chart of the teeth the locations of all fillings and all missing teeth were marked. Each pair of fillings which was studied was connected with a line which in turn was numbered. For each pair the electrically positive filling was marked with a plus sign. The approximate ages of the fillings were also indicated. On the reverse side of the form were four columns headed: Pair Number, V_3, V_1, and V_2; here the observed galvanometer deflections were recorded. The probes were so placed that V_3 always gave a deflection to the right; if any of the other deflections were to the left this was indicated by the use of a minus sign in the recorded deflection.

Results (Part I).—Since the teeth were dry the potential difference V_1 (Fig. 3) was the e.m.f. *(e)* caused by the action of the bone fluid on the two fillings, i.e.,

$$e = V_1.$$

The resistor R_2 was connected from one probe conductor to the other (by closing switch S, Fig. 1), thus closing the electric circuit. Since R_2 was of the order of 1 megohm (1,000,000 ohms) whereas the resistance of the two probe-conductors was only a fraction of 1 ohm, the entire electrical resistance in the circuit could be considered as $R_2 + R_B$, where R_B was the unknown resistance between the two fillings through the bone fluid. Ohm's laws apply to this single-path circuit; they yield the two equations:

$$i_2 = V_2/R_2, \text{ and } i_3 = e/(R_2 + R_B),$$

where V_2 is the measured potential difference between the two probes, i.e., across R_2, and i_2 is the current through R_2 and the bone fluid.

These three equations may be solved for the resistance through the bone fluid in terms of the measured and known quantities. Thus:

$$R_B = R_1 (V_1/V_2 - 1).$$

In the early work many data were found to be useless because of excessive polarization effects which were caused by the use of too large a condenser (C) or too low a resistance R_2. Also the proper technique and care in drying the teeth had not been perfected. In the later work some data were rendered useless because of excessively rapid polarization effects for tiny fillings in some subjects. Usable data were obtained on 213 pairs of fillings in 137 subjects having "normal" mouths. These results are summarized in Table I.

TABLE I

SUMMARY OF RESULTS FOR PAIRS OF FILLINGS

137 INDIVIDUALS 84 MALE; 53 FEMALE	AMALGAM AMALGAM		GOLD AMALGAM		GOLD GOLD	
125 Pairs *Male*	83		32		10	
88 Pairs *Female*	72		13		3	
213 Pairs *Total*	155		45		13	
AVERAGE VALUES	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Potential difference wet, V_2 mv	142	162	207	271	63	91
Bone fluid e.m.f., e						
positive mv	147(80)*	156(69)	215	271	65	101
negative mv	41(3)	30.5(3)	—	—	—	—
Bone fluid resistance R_B megohms	1.30	1.32	0.465	0.89	0.85	0.82

*Number in parentheses means number of pairs of restorations. Positive direction is the same as that of potential difference V_2.

The two greatest observed potential differences (V_2) of pairs of fillings when wet with saliva were 564 and 511 mv. There were six others above 400 mv. The two greatest observed e.m.f.'s (e) caused by the bone fluid were 560 and 510 mv. There were five others above 400 mv.

The pH of each saliva was determined with a paper indicator, the observed values ranging from 6.2 to 7.4. None of the electrical quantities indicated any dependence on the pH of the saliva.

Nearly all the subjects fell in the 18 to 30 age range but there were some in the 35 to 60 range. There was no indication of a dependence of the electrical quantities on age. It also appears that there was no significant dependence on sex.

Five subjects had severe pathologic conditions in their mouths; one had a large white patch on the cheek. Six pairs of fillings were studied in the mouths of three of the patients. These yielded potential differences (V_2) and bone fluid e.m.f.'s (e) which were 2 to 3 times the average for normal subjects. However, a number of subjects having apparently normal oral cavities yielded values of these quantities which were even larger. The other two patients

yielded values which were about half the average values obtained from normal subjects. There was no convincing evidence that any of the lesions were caused by the dental restorations.

A study of the records did not indicate any connection between recent illness and the magnitudes of the measured electrical quantities. The same was true for subjects who reported occasional lesions occurring in their mouths.

An important fact was established by examining the results obtained in oral cavities in each of which two or more suitable pairs of fillings were studied. The three types of pairs chosen were: (1) The two teeth were adjacent or very near each other in the same jaw; (2) the two teeth were in the same jaw but across the mouth from each other; (3) the two teeth were across the mouth from each other but one was above and the other below. More than 20 cases

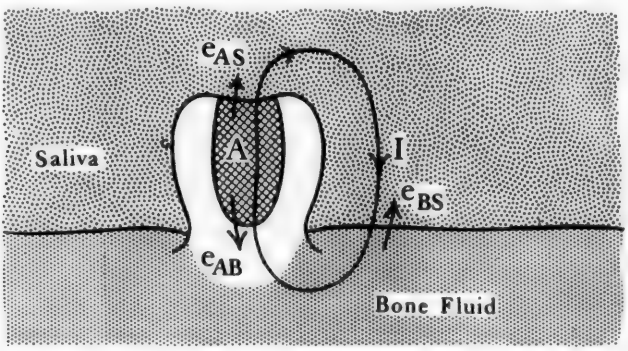

Fig. 4.—An electrical current results when a single metallic dental filling contacts the two electrolytes, saliva and bone fluid, which are themselves in contact.
A, an amalgam filling contacting saliva above and bone fluid below
Net e.m.f. in the circuit is the algebraic sum of e_{AS}, e_{AB}, and e_{BS}, the e.m.f.'s generated at the metal-saliva, metal-bone fluid, and bone fluid-saliva contacts.
I, shows the direction of the net e.m.f. and the resulting electric current

of each of these types were found among our data. The average value of the resistances through the bone (R_B) was calculated for each of the three types of pairs. These three average values indicated that the resistance between a pair of fillings under normal oral conditions is independent of how far apart they are in the mouth. In fact, in several cases the higher resistance was associated with two fillings that were nearest each other. This confirms the long-known fact that the resistance between small electrodes immersed in a large body of electrolyte is concentrated almost entirely in and very near the electrode-electrolyte contacts. Thus it is evident that the electrical resistance associated with a filling in a tooth is concentrated in and very near the metal-saliva and the metal-bone fluid contacts. That part of the resistance out in the bone and the tissues is negligible in comparison.

Part II. Phenomena Associated With Single Fillings.—

Fundamental considerations.—When a piece of metal contacts two different electrolytes and these two electrolytes contact each other, through a porous substance for example, then a net e.m.f. exists in that circuit and an electric current passes through the metal and through the electrolytes.

In Fig. 4 is shown an amalgam filling which contacts both of the electrolytes, saliva, and bone fluid. Since the saliva and the bone fluid are also in contact through tissue, one would expect an electric current through the filling as is indicated by I in Fig. 4.

Fig. 5 shows the electric circuit which is equivalent to that associated with an amalgam filling in an oral cavity containing three other fillings. The significant experimental fact stated above, that the resistances are concentrated at and near the metal-electrolyte contacts, makes this equivalent circuit relatively simple. The resistance and e.m.f. of the amalgam-saliva contact are indicated respectively as R_{AS} and e_{AS}, and those of the amalgam-bone fluid contact as R_{AB} and e_{AB}. The e.m.f. of the bone fluid-saliva contact is shown as e_{BS}. This last contact and the path through the saliva and bone fluid have relatively negligible resistances.

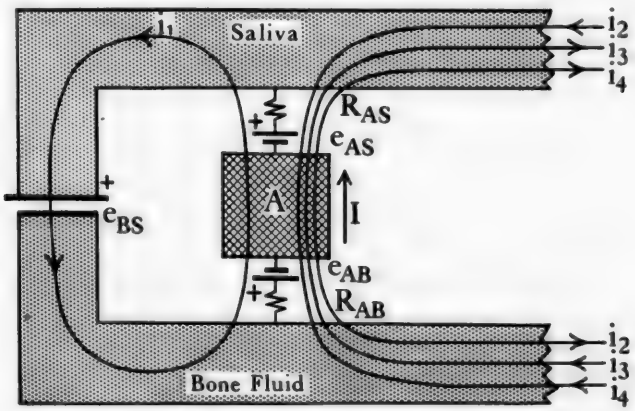

Fig. 5.—Possible electrical phenomena associated with an amalgam dental filling in an oral cavity having three other fillings.

A, an amalgam dental filling

e_{AS} and e_{AB}, the e.m.f.'s of the amalgam-saliva and amalgam-bone fluid contacts

e_{BS}, the e.m.f. of the saliva-bone fluid contact

R_{AS} and R_{AB}, the resistances of the amalgam-saliva and amalgam-bone fluid contacts

i_1 electric current caused by e_{AS}–e_{BS}–e_{AB}.

i_2, i_3, and i_4, currents caused by three other fillings in the same mouth

$I = i_1 - i_2 + i_3 + i_4$, the net current through the filling A

Also in Fig. 5 are shown three other currents i_2, i_3 and i_4 which may pass through the filling. Each of these currents may be caused by A and another filling in that mouth, acting as a pair of fillings in accord with the findings in Part I of this paper. Thus, the total net current (I) which passes up through A is the algebraic sum of the individual currents. Therefore:

$$I = i_1 - i_2 + i_3 + i_4$$

Obviously if any number (N) other fillings exist in that oral cavity, I is the algebraic sum of i_1 and the N other resulting currents (if they exist).

Around any closed path in an electrical network the sum of the electrical potential drops must equal the sum of the electrical potential rises (Kirchhoff's

Law). The closed loop marked i_1 in Fig. 5 is along such a closed path. Thus it is possible to write the following equation:

$$R_{AS}I + R_{AB}I + e_{BS} + e_{AB} = e_{AS}$$

This may be solved for I, thus:

$$I = (e_{AS} - e_{BS} - e_{AB})/(R_{AS} + R_{AB})$$

It is evident that the net e.m.f. in the closed path is $e_{AS} - e_{BS} - e_{AB}$; that the total resistance in that path is $R_{AS} + R_{AB}$; and that I is the total (or net) current that passes through the filling.

It is also clear that R_{AS} and e_{AS} depend only on the natures of the filling and the saliva; that R_{AB} and e_{AB} depend only on the natures of the filling and the bone fluid; that e_{BS} depends only on the natures of the saliva and the bone fluid; and that none of these depends on the presence or absence of other fillings in that oral cavity.

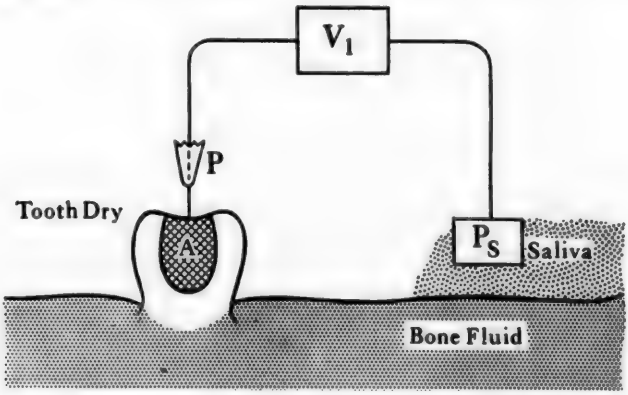

Fig. 6.—The use of the auxiliary electrode for measuring V_1 and V_2 of Part II.
A, an amalgam filling
P, a platinum-tipped probe which contacts the metal filling
P_S, a large auxiliary platinum electrode which contacts the saliva
V_1, the potential difference indicated by a meter which requires no continuous current

Thus the magnitude of the electric current through any metallic dental filling is independent of the number of fillings in that oral cavity, provided that particular filling does not make contact with another metallic filling. The magnitude of this current is given by the last equation above. This conclusion would be exact if the saliva and bone fluid had exactly zero resistance. Their resistance is not zero, but it is very small compared to R_{AS} and R_{AB}. Thus the conclusion above states a very close approximation to the actual situation in an oral cavity.

Theory for Determining the Desired Electrical Quantities (Part II).—Fig. 4 shows the current I which might pass through a metallic filling if that were the only filling in that oral cavity. If other fillings are present the current through the filling tentatively may be represented by I as shown in Fig. 5. It is possible by indirect means to make determinations of the net e.m.f., the

current and the contact resistances. The theory for these measurements will now be developed. In this development it will be assumed that an instrument is available with which electrical potential differences may be measured, without drawing any continuous current from the filling. It will also be assumed that it is possible to keep the tooth entirely free of saliva, and that it is possible to contact only the filling with a tiny drop of saliva so as to leave a dry ring of enamel entirely around the wetted filling.

In Fig. 6 is depicted a tooth which has been well dried and which contacts the bone fluid at its buried surface. The potential difference meter is shown connected to the filling through the platinum-tipped probe (P), and to the saliva through the large auxiliary electrode P_S. Let e_{AB}, e_{BS}, and e_{SP} represent the e.m.f.'s generated respectively at the amalgam-bone fluid, bone fluid-saliva and saliva-platinum contacts. Since the meter uses no current, the potential difference V_1 which it indicates, is the net e.m.f. in that circuit. Therefore:

$$V_1 = e_{AB} + e_{BS} + e_{SP} \quad - - - - - - - \quad [1]$$

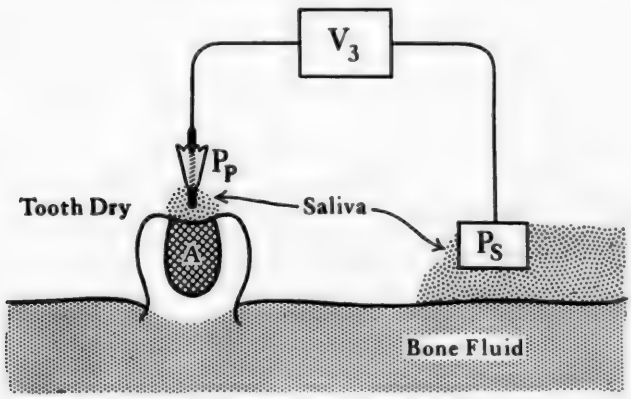

Fig. 7.—The use of the saliva probe and the auxiliary electrode for measuring V_3 and V_4 of Part II.

A, an amalgam filling
P_S, an auxiliary platinum electrode
P_P, a platinum probe which makes an electrical contact with the filling through a tiny drop of saliva.
V_3, the potential difference indicated by a meter which requires no continuous current

When a resistor R_2 is connected from the probe to the auxiliary electrode the e.m.f. in the circuit causes a current i_2 to pass through the resistor. Ohm's laws apply to this single-path circuit and yield the equations:

$$V_2 = R_2\, i_2 \quad - - - - - - - - - - - - - - - - \quad [2]$$

and

$$i_2 = (e_{AB} + e_{BS} + e_{SP})/(R_{AB} + R_2 + R_{PS}) \quad - - - - - - - \quad [3]$$

where R_{AB} and R_{PS} are the resistances respectively of the amalgam-bone fluid and the platinum-saliva contacts. (It was shown in Part I that almost the entire resistance exists at the metal-electrolyte contacts.)

In Fig. 7 is shown the same setup as in Fig. 6 except that the platinum-tipped probe now does not contact the metallic filling directly but does make electrical contact with it through a tiny drop of saliva; the enamel surrounding the filling is kept dry. Here again the potential difference meter indicates (V_3) the net e.m.f. in that circuit. Thus:

$$V_3 = e_{AB} + e_{BS} + e_{SP} - e_{PS} + e_{SA} \quad [4]$$

where e_{PS} and e_{SA} are respectively the e.m.f.'s generated at the probe-saliva and the saliva-amalgam contacts.

When a resistor R_4 connects the auxiliary electrode and the saliva-probe electrode a current i_4 passes through the resistor. Here again Ohm's laws apply and yield the equations:

$$V_4 = R_4\, i_4 \quad [5]$$
$$i_4 = (e_{AB} + e_{BS} + e_{SP} - e_{PS} + e_{SA})/(R_{AB} + R_{PS} + R_4 + R_{PP} + R_{AS}) \quad [6]$$

where R_{PP} and R_{AS} are respectively the resistances of the probe-saliva and amalgam-saliva contacts.

The current in the normal oral cavity is shown in Figs. 4 and 5 and is given by:

$$I = (e_{AB} + e_{BS} + e_{SA})/(R_{AB} + R_{AS}) \quad [7]$$

The e.m.f., which may be introduced by the auxiliary electrode and the saliva-wet probe, occurs in equation [4] as $(e_{SP} - e_{PS})$. This can be measured directly by inserting the probe in the saliva near the auxiliary electrode in the mouth and measuring the resulting potential difference V_P with the meter. Thus:

$$V_P = e_{SP} - e_{PS} \quad [8]$$

The magnitudes of the saliva-probe and the saliva-electrode resistances, R_{PP} and R_{PS}, can be measured by a special electric circuit which will be described in the next section. For the present development these two resistances will be considered to be known quantities.

These eight independent equations state relations involving the unknown quantities, R_{AB}, R_{AS}, net e.m.f., I, $(e_{SP} - e_{PS})$, e_{SP}, i_2, and i_4, and the measured and known quantities V_1, V_2, V_3, V_4, V_P, R_2, R_4, R_{PS}, and R_{PP}. The solutions of these equations for the desired electrical quantities, R_{AB}, R_{AS}, net e.m.f., and I are:

$$R_{AB} = (V_1/V_2 - 1)\, R_2 - R_{PS} \quad [9]$$
$$R_{AS} = (V_3/V_4 - 1)\, R_4 - R_{AB} - R_{PS} - R_{PP} \quad [10]$$

(In the calculations the numerical value of R_{AB} from [9] would be used in [10].)

$$\text{Net e.m.f.} = V_3 - V_P \quad [11]$$
$$I = (V_3 - V_P)/(R_{AB} + R_{AS}) \quad [12]$$

(In the calculations, values from [9] and [10] would be used for R_{AB} and R_{AS}.)

R_{AB} is the resistance of the metal-bone fluid contact of the filling.

R_{AS} is the resistance of the metal-saliva contact of the filling.

Net e.m.f. is the algebraic sum of the e.m.f.'s generated at the metal-bone fluid, metal-saliva and bone fluid-saliva contacts.

I is the electric current which passes through a metallic filling in an oral cavity under ordinary oral conditions, regardless of the number and kinds of other fillings in that oral cavity.

Apparatus (Part II).—The condenser-ballistic-galvanometer potential-difference meter, described in Part I, was used for many measurements. However, it was found that the 1-mf condenser took so long to charge (5 sec) that the current through the resistor (R_2 or R_4) often caused intolerable polarization effects. These were indicated by the lack of reproducibility of the potential difference measurements when the resistor (R_2 or R_4) was being used. In fact, the reproducibility of the measurements made on single fillings was much poorer than that found in Part I where the apparatus was used with pairs of fillings. A smaller condenser would have used less charge and would have had a shorter charging time, but a sufficiently small condenser would not take enough charge to operate even a very sensitive ballistic galvanometer.

This difficulty was overcome by designing and constructing what is essentially a direct current amplifier which uses the recently developed Victoreen 5803 electrometer tube. With this tube a condenser of only 0.01 mf is required, for which the charging time is only a small fraction of a second. This short closed-circuit time made it possible to use a 0.25 megohm resistor for R_2 and R_4, and thus to increase the accuracy of the resistance determinations. This electrometer apparatus required a very highly insulated quadruple-contact key, two very highly insulated switches, highly insulated wires in the grid circuit, and a polystyrene base for the electrometer tube. Polystyrene rods and amphenol sheets and tubes furnished sufficiently good insulation. The steady deflections were indicated on a Leeds & Northrup Type "P" galvanometer. A circuit for calibrating the meter at hundred-mv intervals up to 600 mv, and at 800, 1000, 1200, and 1400 mv, was incorporated in the instrument. This instrument is entirely battery operated, and, after a warm-up period of a half hour, retains its "zero" and calibration for several hours without adjustment. It will be described in detail in a separate publication. All potential difference measurements were made with this vacuum-tube electrometer apparatus.

The platinum-tipped probe used for contacting the fillings for the potential difference measurements V_1 and V_2 as indicated in Fig. 6 was the same probe that was used in the experimental work described in Part I; its construction is shown in Fig. 2.

The construction of the tip of the saliva probe used in the measurements of V_3 and V_4 as indicated in Fig. 7, is shown in Fig. 8. The tip of this probe was dipped into the saliva in the mouth and then touched to the metallic filling so that the polystyrene pin, P_3, rested on the filling. The 15 wires W_3 made a large area of contact with the tiny drop of saliva which was held by surface tension and which also was in contact with the filling.

It was necessary to have the leads to each of these probes and to the auxiliary electrode exceedingly well insulated, and well shielded with a grounded metal sleeve. Shielded lapel-microphone cable having polyethelene insulation was found to be entirely satisfactory.

The auxiliary electrode, P_S, in Figs. 6 and 7, was made of 4 inches of $\frac{1}{16}$ inch diameter platinum wire. A $2\frac{1}{2}$ inch length of this wire was wound into a tight flat spiral, the outside turn of which had a diameter of $\frac{1}{2}$ inch; the remainder served as a lead out of the mouth where it joined the flexible copper lead in the shielded microphone cable. This junction was covered with gum-rubber tubing which was cemented to the platinum and to the cable with gum-rubber cement. This spiral electrode was placed in the saliva under the tongue at the side of the mouth.

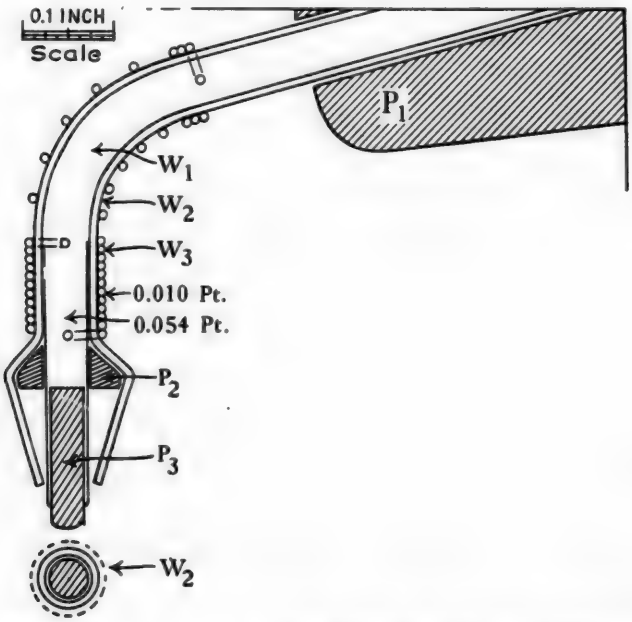

Fig. 8.—Construction of the tip of the saliva probe used for making a saliva contact with the metal filling while leaving a ring of dry enamel surrounding the filling

W_1, 0.054 inch platinum wire
W_2 and W_3, 0.010 inch platinum wire
P_1, tapered end of half-inch polystyrene rod; this handle is like that for the probe described in Part I
P_2, a polystyrene washer
P_3, a 0.035 inch polystyrene pin which contacts the dental filling.

The circuit for measuring the resistance R_{PS} of a spiral auxiliary-electrode-saliva contact is shown by the schematic diagram, Fig. 9. Two exactly similar spiral electrodes were immersed in the saliva under the tongue in a

subject's mouth. Since there were two electrode-saliva contacts the resistance between the two through the saliva was $2R_{PS}$. For this circuit it is possible to write three independent equations. These are:

$$R_o i_o = V \quad - - - - - - - - - - - - - - - - \quad [13]$$
$$R_b i_b + R_a(i_b - i_o) = e_b \quad - - - - - - - - - \quad [14]$$
$$2R_{PS} i_o + R_o i_o - R_a(i_b - i_o) = e_o \quad - - - - - - - \quad [15]$$

Substituting for i_o and i_b in equation [15] from equations [13] and [14], we have:

$$2VR_{PS} = R_a R_o e_b/(R_a + R_b) - R_o V + R_a^2 V/(R_a + R_b) - R_a V + R_o e_o \quad - - \quad [16]$$

If the reversing switch in Fig. 9 is reversed then all currents will be reversed, all resistances will remain the same, and e_o will not change in magnitude or direction because it depends only on the natures of the two electrodes and the saliva. Consequently an equation exactly like [16] will result except that the last term, $e_o R_o$, will be negative.

If V and V' are the observed values of the potential differences for the two positions of the reversing switch, the addition of the two equations like [16] eliminates the term containing the e.m.f. e_o of the electrodes. The resulting equation is:

$$2R_{PS} = 2\ R_a R_o e_b/(V + V')(R_a + R_b) - R_o - R_a + R_a^2/(R_a + R_b) \quad - - \quad [17]$$

Since R_o was 0.25 megohm, whereas R_a was 300 ohms and R_b was approximately 1200 ohms, the last two terms are very small compared to the first two on the right hand side of the equation [17]. These last two terms therefore may be neglected. The resulting equation is:

$$R_{PS} = R_a\ e_b\ R_o/(V + V')(R_a + R_b) - 0.5\ R_o \quad - - - - - - \quad [18]$$

R_a, R_b, and e_b were adjusted so that the term, $R_a e_b/(R_a + R_b)$ was 300 mv. Thus the resistance R_{PS} of a spiral electrode-saliva contact was given by equation [18] when V and V' were expressed in millivolts.

The resistance R_{SP} of a saliva-probe contact (Fig. 8) was measured with this same apparatus.

Experimental Procedure (Part II).—Students in the School of Medicine, and graduate and student nurses, all in the 18 to 30 year age group, served as subjects in this study of electrical phenomena associated with single metallic fillings. Apparently, all had normal oral cavities.

Since it was necessary to maintain a ring of dry enamel entirely around each filling in the measurements depicted in Fig. 7 (both without and with R_i) in which only the filling made a saliva contact with a platinum probe, those fillings were selected which were situated near the centers of the teeth.

Each potential-difference measurement was repeated four times. This was feasible because of the rapidity with which the vacuum-tube electrometer could be operated. This repetition made it possible to detect polarization effects since these made reproduction of results impossible. Very little difficulty with polarization effects was encountered, but the inability to keep a

ring of dry enamel entirely around a filling, while measuring V_3 and V_4 (Fig. 7), did cause the discarding of many sets of data. It just was not possible to find one or more ideally located fillings in each oral cavity.

Since polarization effects were usually negligible when the vacuum-tube electrometer was employed to measure the potential differences, the order of taking the potential differences was V_1, V_2, V_3, and V_4, and V_P. If a second filling in that oral cavity was investigated, measurements of V_P were not made again since it was found that they duplicated those made after the measurements on the first tooth.

The electrical measurements were carried out with the subject in a reclined position in a barber chair. The platinum spiral auxiliary electrode P_S was placed under and to the side of the subject's tongue. An oral pack was put in place and the selected tooth, together with the adjacent teeth, were dried carefully with the ethyl-alcohol-air-blast method described in Part I. While one research assistant pressed the platinum-tipped probe (P) (Fig. 6) on the filling, the second assistant pressed the quadruple-contact key of the vacuum-tube electrometer, released it immediately, and read and recorded the steady deflections of the galvanometer. This measurement was repeated 3 more times.

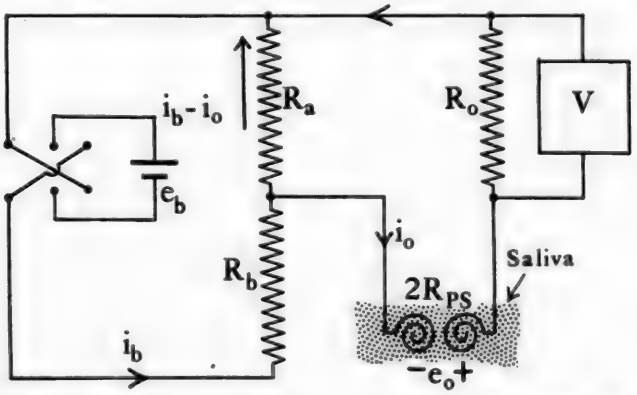

Fig. 9.—Schematic diagram of the electric circuit used to measure the resistances introduced by the auxiliary electrode and the saliva probe.
e_b, e.m.f. of dry cell connected through a reversing switch
R_a and R_b, Leeds & Northrup resistance boxes
$2R_{PS}$, the resistance between the two spiral auxiliary electrodes in an oral cavity
e_o, the e.m.f. caused by the possible dissimiliarity of the spiral electrodes; usually a few mv.
R_o, a 250,000 ohm resistor
V, the potential difference indicated by the electrometer-tube meter which uses no current

A resistor (R_2) of 0.25 megohm was connected across the probe and electrode leads, and the potential difference (V_2) was measured: this was repeated 3 more times.

The platinum-tipped probe was replaced with the saliva probe (P_P) (Fig. 7). This saliva probe was immersed in the saliva in the mouth until its tip was covered with a small drop. It was then pressed on the filling so that only the polystyrene pin (P_3, Fig. 8) touched the filling. The potential difference

V_3 was then measured 4 times. A resistor (R_4) was connected across the probe and electrode leads and the potential difference (V_4) was measured 4 times.

It is nearly impossible to get the surfaces of two pieces of platinum (or any metal) so nearly alike that they will not generate an e.m.f. when they are placed in an electrolyte (saliva). This possible e.m.f. (V_P) is indicated in equation [8], and was determined by measuring the potential difference between the saliva probe (P_P) and the auxiliary electrode (P_S) when these were near each other and both immersed in the saliva in the mouth. This measurement also was repeated 4 times.

The form for recording these data included all the items described for the form used in Part I except that the columns were headed V_1, V_2, V_3, V_4, and V_P. These potential differences were recorded as galvanometer deflections in millimeters. Usually measurements on two fillings were made in each oral cavity.

The resistance (R_{PS}) of the auxiliary-electrode-saliva contact was determined with the apparatus shown schematically in Fig. 9 and the use of equation [18]. It was found that R_{PS} was independent of the relative locations of the two auxiliary electrodes in the mouth. It was also found that this resistance was virtually the same when it was measured in the mouths of various people. Since it was small compared to the resistance of a filling-saliva or a filling-bone fluid contact, R_{PS} was considered a constant for the apparatus. Its measured value was 0.007 megohm.

The resistance (R_{PP}) of a saliva-probe contact was also determined with the apparatus shown in Fig. 9 and by the use of equation [18]. In this case a pair of identical saliva probes replaced the spiral electrodes in Fig. 9. These two probes were dipped into the saliva in the subject's mouth, and their tips were brought together out in the air so that the ends of the two polystyrene pins (P_3, Fig. 8) touched each other and so that the two tiny drops of saliva coalesced. Here again it was found that R_{PP} was essentially the same when determined with the use of saliva drops obtained from various oral cavities. It also was found that R_{PP} was independent of the size of the coalesced drops which connected the two probes. Since R_{PP} also was rather small compared to the resistances of the saliva-filling and bone fluid-filling contacts, it was considered an apparatus constant. Its measured value was 0.028 megohm.

From the data obtained for V_1, V_2, V_3, V_4, V_P, R_2, R_4, R_{PS}, and R_{PP}, were calculated the values of the four desired quantities, R_{AB}, R_{AS}, net e.m.f., and I by use of equations [9], [10], [11], and [12]. These calculations were carried out conveniently by use of a record form having 14 columns. The galvanometer deflections in millimeters were translated into potential differences in millivolts before recording them on this form. The electrometer apparatus was adjusted to a sensitivity of 2.5 mv/mm for these measurements.

Results (Part II).—Usable data were obtained on 78 fillings in the oral cavities of 66 subjects whose ages fell in the range 18 to 30 years. The results are summarized in Table II in which are given average values of the desired electrical quantities.

The magnitudes of the three largest observed values of each of the electrical quantities are: For Gold: R_{AB}:0.31, 0.19, 0.14; R_{AS}:1.4, 1.3, 0.87; (R_{AB} + R_{AS}) :1.5, 1.3, 0.90; I:1.07, 0.44, 0.24; *e.m.f*:245, 160, 150. For amalgam: R_{AB}:0.42, 0.31, 0.30; R_{AS}:1.7, 1.5, 1.3; (R_{AB} + R_{AS}) :2.0, 1.6, 1.3; *e.m.f.*:160, 151, 145; I:3.4, 2.8, 1.9.

TABLE II

SUMMARY OF RESULTS FOR SINGLE FILLINGS

78 Fillings in Mouths of 66 Persons

Average Values—Part II

78 FILLINGS	66 AMALGAM	12 GOLD
Resistance of the bone fluid contact, R_{AB}	0.067 megohm	0.097 megohm
Resistance of the saliva contact, R_{AS}	0.24 megohm	0.44 megohm
Total resistance	0.31 megohm	0.54 megohm
Net e.m.f. associated with filling		
Positive*	87 mv. (29)**	67 mv. (7)
Negative	54 mv. (37)	136 mv. (5)
Normal electric current through filling, I		
Positive*	0.48 microamp. (29)	0.30 microamp. (7)
Negative	0.48 microamp. (37)	0.22 microamp. (5)

*Positive direction of e.m.f. and current is from filling to saliva to bone to filling.
**In parentheses is given the number of fillings used to compute the average value.

The result that the resistance between a pair of platinum spiral auxiliary electrodes was independent of their relative positions in the oral cavity, also shows that the resistance introduced by such an electrode is concentrated in, and very near, the metal-saliva contact, and that the resistance offered by the saliva, bone and tissues is relatively negligible.

The similar result that the resistance between a pair of saliva-probes (Fig. 8) was independent of the size of the drop of saliva which connected them, also shows that the resistance introduced by such an electrode is concentrated at, and near, the metal-saliva contact.

Both of these results corroborate the experimental fact noted in Part I, that the resistance between a pair of fillings was independent of their relative positions in the oral cavity, which led to the conclusion that the resistance limiting the normal current through a filling is concentrated at and near its contacts with the saliva and the bone fluid.

While the electrometer-tube potential-difference meter was being constructed and perfected, many data on single fillings were taken with the aid of the ballistic-galvanometer-condenser potential-difference meter which is described in Part I. With this meter usable data were obtained on 127 fillings in the mouths of 77 persons. Eleven of the fillings were gold, 116 were of amalgam.

Since the charging time for the one-mf condenser was 5 seconds, serious polarization effects were present in spite of the fact that a 2 megohm resistor served for both R_g and R_i. The measurements of the net e.m.f. did not involve the use of current through the resistor, and these measurements agree very well with those obtained with the electrometer-tube apparatus. In fact in both sets

of measurements the average value of the positive e.m.f. associated with an amalgam filling was 87 mv. The average negative e.m.f. for amalgam was 54 mv for the electrometer data and 60 mv for the ballistic galvanometer data. The number of gold fillings in each set was too small (5 to 7) to yield good agreement; they were respectively 67 and 136 mv, and 161 and 46 mv. This is an entirely satisfactory agreement for such small numbers of measures on such highly variable quantities.

The resistance measurements involved the use of current and the resulting polarization effects (with ballistic galvanometer apparatus) evidently caused the resistances of the metal-saliva and metal-bone fluid contacts to increase (or the existing e.m.f.'s to decrease, which would give the same effect). In fact the average computed resistances were several times those computed from electrometer data. The average currents were correspondingly less by the same factor. Again, these results may be considered in satisfactory agreement for they disagree by less than half an order of magnitude. This is far less disagreement than may occur between sets of measurements on two different fillings in the same oral cavity. Serious polarization (decrease in e.m.f. or increase in resistance, or both) was evidenced by the fact that successive values of both V_2 and V_4 decreased in size. This occurred very seldom with the electrometer apparatus.

Considerations of the results obtained with pairs of fillings in Part I, in the light of the results obtained with single fillings, are of interest. One also would expect that R_B of Part I, which is made up of two filling-bone fluid contacts, should be about twice as large as R_{AB}. Actually the former is some 5 times larger. This probably is due to the polarization effects which occurred when the resistor (R_2) was connected for the 5 second intervals and a 1 mf condenser was charged.

A few of the subjects reported electric shocks when two of their fillings were contacted with the probes (connected to the 1 mf condenser); evidently even the small charge taken by the condenser was sufficient to cause this disagreeable sensation. If two fillings, wet with saliva, are connected through an ordinary microammeter or galvanometer, both the e.m.f. caused by the saliva and the e.m.f. caused by bone fluid (acting on the pair of fillings) furnish current through the meter. Early workers reported such currents to be as much as 50 microamperes, and even much more in some cases. These facts indicate that contacts between metallic fillings in place in teeth, must be avoided. If necessary a plastic plug may be inserted in one of them at the point of contact.

The many case histories that have been reported in the literature lead one to conclude that serious pathologic conditions in the oral cavity have been caused by metallic dental fillings. There appears to be no evidence that such conditions are caused directly by the electric current. However, if the subject happens to exhibit hypersensitivity to certain metallic ions supplied by the dental fillings, then, since the electric current hastens the solution of the fillings and assists in transporting the ions to and through the tissues, it may exert an indirect detrimental effect on the subject.

The mere size of the electromotive force probably is not significant. Three of the five patients who had decided pathological conditions in their mouths exhibited electromotive forces much larger than the average from "normal" subjects, but quite a number of the subjects with apparently "normal" mouths exhibited even larger electromotive forces. However, the number of cases (5) exhibiting decided pathological conditions was too small to permit the drawing of final conclusions.

CONCLUSIONS

A critical survey of the literature since 1878 together with the results obtained in the present work, lead to the following conclusions:

1. There appears to be good evidence that serious pathologic conditions in the oral cavity have been caused by metallic dental fillings.

2. The mere size of the electromotive forces associated with metallic fillings is probably not of primary significance (this conclusion is based on only a few observations). A more important factor may be the hypersensitivity of the subject to the metallic ions supplied by the fillings.

3. Relatively large electric currents will pass through metallic fillings if two such fillings are in contact. Such contacts between metallic fillings should be prevented.

4. The magnitudes of the electrical phenomena associated with metallic dental fillings do not appear to depend on the pH of the saliva, on the sex or age of the subject, or on the existence or nature of recent illness.

5. In this report are shown: (a) that the bone fluid is just as important as the saliva in causing electric currents; (b) methods for determining the net e.m.f.'s associated with metallic dental fillings; (c) methods for determining the electrical resistances associated with metallic dental fillings; (d) that these resistances are concentrated at the metal-saliva and metal-bone fluid contacts; (e) a method for determining the net electric current passing through a metallic dental filling under ordinary oral conditions; and, (f) that the magnitude of the current through any filling is virtually independent of the number and kinds of other fillings in that oral cavity, provided that filling does not make contact with any other metallic filling.

SUMMARY

A critical review of the literature from 1878 to date is presented. The more important experiments are described and the more important conclusions are cited. Certain fallacies are pointed out.

The sources of possible electromotive forces for pairs of metallic fillings and for single fillings are described. The literature survey revealed that neither these e.m.f.'s, nor their resulting currents, had been measured, and that no measurements had been reported from which these e.m.f.'s and currents could have been calculated.

A condenser-ballistic-galvanometer potential-difference meter was developed, with which the electrical potential differences between pairs of metallic fillings could be measured. The scheme for carrying out the measurements and the theory for the calculations are given.

Measurements were made on 213 pairs of fillings in the mouths of 137 subjects. Average values of the results are given in Table I.

A study of these measurements (Part I) led to the conclusion that the electrical resistance associated with a filling is almost entirely concentrated in, and very near, the metal-saliva and metal-bone fluid contacts. This was confirmed in Part II for metal-saliva contacts.

This conclusion made it possible to prove, by use of the well-known principles applying to electric circuits, that the electric current through any metallic filling is virtually independent of the number and kinds of other metallic fillings in that oral cavity, provided that filling is not in contact with any other metallic filling.

This proof, together with the conclusion above, made it possible to develop a method, including the necessary theory, for determining the electromotive force, electrical resistance, and electric current associated with a single metallic filling. The theory calls for the measurement of five potential differences as well as the resistances introduced by an auxiliary electrode and a saliva probe.

A second apparatus for measuring the potential differences was developed: it incorporated a special highly-insulated electrometer tube. A potential measurement with this meter required a closed-circuit time of only a fraction of a second, and only a very small electric charge needed to be furnished by the source of the potential. This meter eliminated intolerable polarization effects.

The experimental procedure is described and the scheme for dealing with the data is given. Results were computed for 78 fillings in the oral cavities of 66 subjects whose ages fell in the range 18 to 30 years. These results are summarized in Table II.

These results are discussed and their relations to other results obtained with the condenser-ballistic-galvanometer meter on 127 fillings in the mouths of 77 persons, are described.

One of the authors (Schriever) is indebted to Dr. E. S. Lain of Oklahoma City for introducing him to this problem, for inviting him to cooperate in some early work (Reference No. 4), and for loaning him a collection of reprints and some private correspondence.

Both authors are indebted to Dr. Onis Hazel of Oklahoma City for referring to us five of his patients who exhibited severe pathologic conditions in their mouths; and to Constance Raab, Loretta Graham, Charles Engles, Arthur W. Buswell, and Leo Cawley, research assistants, for doing much of the routine experimental work.

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A STUDY OF THE HEALING PROCESS FOLLOWING OPERATIVE INTERFERENCE WITH THE CONTINUITY OF THE RAT MAXILLA

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THE OBJECT of the present study is to determine what type of repair occurs in the maxilla of the rat following operative interference with the continuity of the bone.

There is general agreement among investigators concerning the mechanism of bone repair for most of the bones of the body. A great deal of information is available concerning fracture of the mandible because of its size, form, relatively exposed position, and frequency of fracture. Sarnat and Schour[13] have shown that when the mandible of a rat is fractured experimentally, it heals by bony union. Furthermore, it has been demonstrated that even though the mandible is a membrane bone, cartilage formation can be detected in the callus during the repair process following fracture.

The maxilla, which is also a membrane bone, is more complex, less frequently fractured, and occupies a less exposed position than the mandible. Consequently, the bulk of information on maxillary fractures deals largely with methods of treatment, and experimental studies designed to determine the type and manner of repair following fracture have been neglected.

Whereas investigators and clinicians generally agree that the mandible heals by bony union following fracture, there is no unanimity of opinion as to the type of repair of the maxilla following fracture. According to one view the maxilla is similar to the flat bones of the skull in that it usually heals only by fibrous union when fractured. The opposing view holds that the maxilla, like the mandible, is capable of healing by bony union. The present study, therefore, was undertaken to ascertain whether the maxilla of the rat, following experimental removal of a thin segment of bone to simulate fracture, heals by fibrous or by bony union.

REVIEW OF THE LITERATURE

Healing of a Typical Long Bone (Tibia).—There is complete agreement among investigators concerning the method of repair of fractures of the long bones, and there is adequate experimental data to warrant acceptance of the current literature. Urist and McLean[18] differed with the earlier authors, Ham[8] and McKeown and Downs,[10] only in their interpretation of the origin of the various cellular elements and the significance of the periosteum and endosteum.

Enneking[6] in an excellent experimental study based on complete fractures of rat tibias described the immediate effect of a fracture as being the formation of a hematoma at the site of the injured tissues. Layer on layer of this blood clot becomes organized and replaced by granulation tissue which, in turn,

This paper was condensed from the thesis presented for partial fulfillment of the requirements for the degree of Master of Science in Anatomy at The Ohio State University, June, 1950.

Received for publication, Oct. 19, 1951; revised by author, Feb. 15, 1952.

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shows differentiation into fibrous connective tissue. The next stage is the development of hyalin and fibrocartilage from the fibrous connective tissue. Bone from either fragment grows into the whole mass of fibrocartilaginous callus between the fracture ends. Union occurs when the growth of bone projected from one fragment meets that from the other and is reorganized between the cortical ends so as to repair the shaft.

Healing of Mandible.—Healing of the mandible following fracture has been excellently described by Grimson,[7] Urist and Johnson,[17] and Sarnat and Schour.[18] These studies describe the healing process, starting with the hematoma and describe its subsequent organization into fibrous connective tissue. This stage is named "the stage of procallus" and is similar in nature to the healing of soft tissue elsewhere in the body. The next stage is that of fibrocartilaginous callus. In this stage the connective tissue shows evidence of the formation of a cartilaginous disc separating the two fragments. The last stage is the gradual replacement of the fibrocartilaginous callus by bone and architectural reconstruction of this newly formed bone. According to Sarnat and Schour,[18] in the healing of the mandible in the rat there is usually a central body callus and occasionally an external callus, but no internal callus. Urist and Johnson[17] pointed out that in tubular bone (diaphysis) healing of the fracture is accompanied by an external and an internal callus in addition to the central callus, but that in fractures in the region of the metaphysis there is only the simple central callus, and healing occurs by the formation of intramembranous bone. The reaction in the mandible, therefore, is comparable to that seen in the metaphysis.

Healing of a Typical Flat Bone.—There has existed in the past considerable disagreement as to the manner of healing of fractures of the bones of the skull. Most of the reports in the literature have been based on clinical observations in the human being, and the lack of exact knowledge concerning the processes involved was due to the paucity of histological observations on experimental material. Sicher and Weinmann[14] have adequately summarized the current knowledge bearing on this type of healing. They admit that fractures of the bones of the skull, especially of the vault, show retarded and reduced callus formation and, quite frequently, the end result is not bony union but a persistent dense fibrous connective tissue union. Sutro and Jacobson[15] were the first to experimentally fracture the parietal bone of the rat and to examine histologically the type of union. The initial findings were a considerable amount of hemorrhage, and the later replacement of this by a membrane of fibrous connective tissue, the cells of which were very sparse. The fractured ends exhibited localized necrosis and proliferation of the periosteal membrane. No true callus was encountered in any of their sections. They also mentioned that cartilage did not develop. They asserted that the defects were closed by bony bridges which were the result of a steady progression of new bone formation through the intermediation of osteoblasts from the broken edges.

Healing of Maxilla.—There exists in the literature to date definite confusion as to the method and end results of healing of the maxillary fractures. Such authors as Wilensky[19] and Clark[3] have held that fractures of the maxilla

usually undergo firm fibrous union unless fixation is lacking and some mechanism is present that produces constant movement of the detached segment. Wilensky[20] explained the absence of bone union in maxillary fractures as being due to the maxilla's lack of a periosteal membrane. The basis for this argument was refuted by Edwards and Kitchin[5] by their histologic sections of a cadaver maxilla. Colyer and Sprawson[4] stated that the maxilla always heals by fibrous union. Meade[11] and Brayshaw[2] described the normal healing of maxillary fractures to end in bony union. The absence of a cartilaginous phase during callus formation, which has been observed frequently, has been explained on the basis of differences in the ontogenesis of bones. Cartilaginous callus was thought to be absent in bones which develop intramembranously. However, this is incorrect since Sicher and Weinmann[14] have shown that the cartilaginous callus is frequently absent in the healing of fractures of the body of the scapula (an endocondral bone) and Sarnat and Schour[13] have shown that cartilaginous callus is present in the healing of experimental fractures of the mandible (a membrane bone).

MATERIALS AND METHODS

The animals used in this study were 6 male albino rats which were subjected to operative interference with the continuity of the maxilla. The site of operation was identical in location in all animals. Careful consideration was given to the selection of the area of operation, in order that it would be easily demonstrable radiographically in the living animal. Two pilot series of 6 animals each were observed, and the area of choice was determined to be the zygomatic process of the maxilla.

Using clean, but not necessarily sterile technique, the animals were etherized and the oral mucosa incised over the area of the right zygomatic process of the maxilla. A No. 700 tapered fissure dental burr and a dental handpiece with motor were employed to cut a slot approximately halfway through the zygomatic process extending anteriorly from the free edge of its posterior border. The defect created did not completely sever the bone into two fragments, and consequently the factors of fixation and apposition of the two edges were not variables to be considered in this study. In order to avoid excess heat production and accompanying bone necrosis, only new burrs were used, and the revolving speed was the lowest possible to produce the defect. No sutures or antibiotics were used following the operation. The animals were placed on a milk diet for 48 hours after which they were allowed the regular laboratory feed (Purina Laboratory Chow).

The animals were sacrificed at intervals of 6 weeks, 9 weeks, 12 weeks, 4 months, 5 months, and 6 months.

Each of the animals was radiographed before and after surgery and following sacrifice. Ordinary dental periapical films were used with 4 seconds' exposure and a target distance of 10 cm. between the x-ray tube and the object. The animals were cancelled with the use of ether in a closed bell jar, and

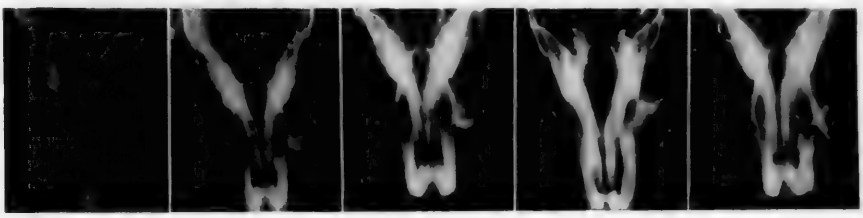
following decapitation the entire heads were placed in 10 per cent formalin. Seventy-two hours were allowed for fixation, and then the skin was dissected away to facilitate more rapid decalcification and infiltration.

a *b* *c* *d* *e*

Fig. 1.—Radiographs of the first five experimental rat skulls prior to surgery.

a *b* *c* *d* *e*

Fig. 2.—Radiographs of the first five experimental rat skulls following surgery.



a *b* *c* *d* *e*

Fig. 3.—*a*, After 6 weeks' healing. *b*, After 9 weeks' healing. *c*, After 12 weeks' healing. *d*, After 4 months' healing. *e*, After 5 months' healing. ×1.

The decalcification technique of Morse,[9] using Custer's solution, was chosen in order to minimize the distortion and maceration so commonly found with nitric acid. Approximately 30 days were allowed for complete decalcification, and the decalcifying solution was changed every other day. At the end of the 30-day period, or when the decalcifying solution tested negatively for calcium ions, the heads were washed in running water for 48 hours and dehydrated through a graded series of alcohols and alcohol-ether. Five concentrations of celloidin (2, 4, 6, 10, and 15 per cent) were used for infiltration, and 48 hours in the incubator at 37° C. were allowed for each concentration. The heads were imbedded in 15 per cent celloidin, hardened in 70 per cent alcohol, mounted on

pressed fiber blocks and cut serially in frontal sections at 18 micra. The sections were stained with hematoxylin and eosin and mounted on sildes with Clarite.

OBSERVATIONS

Clinically, the animals used for the first four healing stages made uneventful recovery from the operations. No infection was evident in the area of the incision, nor were there any external draining sinuses. The animals used for the fifth and sixth stages showed first the symptoms of acute and then chronic infection. External draining sinuses were present under the right orbit. There was a loss in facial cheek tissue more noticeable on the right side. There was a definite loss in total body weight, and the animals were not as active as their fellow experimental animals or controls.

Microscopic examination reveals the malar process of the maxilla of the control animals to be composed of thin cortical bone in the anterior one-third (Fig. 4). In the posterior two-thirds the bone becomes progressively thicker retaining the thin cortical layers peripherally with a central core of a more cancellous type of bone with numerous small marrow spaces.

The first animals was sacrificed 6 weeks postoperatively and the radiograph reveals a definite minimal amount of filling in of the defect (Fig. 3). Microscopic examination shows the line of incision to be covered with epithelium and is seen as a projection from the oral cavity in these frontal sections (Fig. 5). There is a small spicule of bone sequestrated through this site of the incision, and this entire area gives evidence of chronic inflammation by the round cell infiltration. The defect is filled with fibrous connective tissue of a mature character. The bone on both edges of the defect shows osteoblastic activity with the laying down of osteoid tissue. Some of the new bone laid down has already been calcified, and the architectural pattern is cancellous in nature with numerous cells and relatively few Haversian systems. The cancellous projections of new bone are separated by the mature connective tissue resembling the bone marrow. The periosteum is thickened and shows increased osteoblastic activity.

The radiograph at the 9 weeks' stage of healing shows a definite filling in of the defect with the consequent diminution in the size of the radiolucent slot (Fig. 3*b*). Histologically a thin layer of new bone is bridging the defect in the more anterior region with the uniting of the spicules from the two edges (Fig. 6). There is a marked increase in thickness of the bone on either side of the new bridge, and the periosteum is thicker and shows more activity than the control side. The incision is completely covered with epithelium, and the projection from the oral cavity noted in the 6 weeks' stage is not present. In the more posterior region the histologic picture is identical with that described under the 6 weeks' stage.

The operative defect is approximately two-thirds obliterated at the end of 12 weeks as evidenced by the radiograph (Fig. 3*c*). Histologically the defect is bridged by calcified new bone. The entire bone shows an increase in thickness over the control side with a central thin core of compact bone sur-

Fig. 4. Fig. 5. Fig. 6.

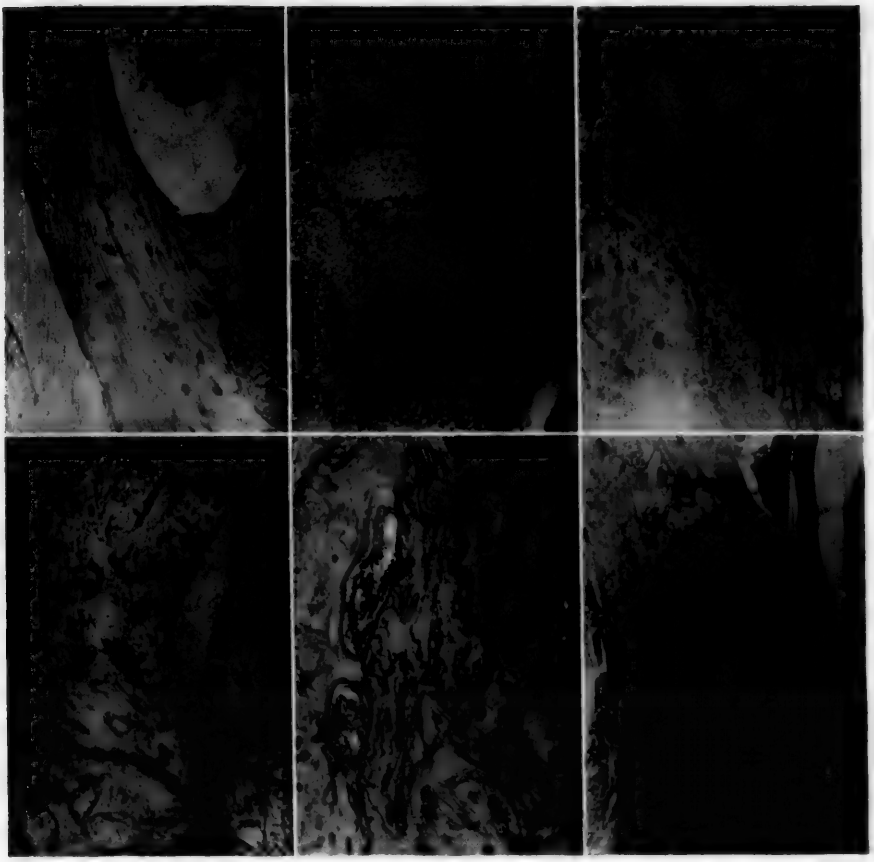


Fig. 7. Fig. 8. Fig. 9.

Fig. 4.—Normal control zygomatic process. ×75.

Fig. 5.—Connective tissue callus at the 6 weeks' healing stage. ×75.

Fig. 6.—New bridging bone found in the 9 weeks' healing stage. ×75.

Fig. 7.—Healing stage at 12 weeks. Note increase in thickness of the bridging bone as compared to Fig. 6. ×75.

Fig. 8.—Healing stage at 4 months. Note architectural rearrangement as compared to that in Fig. 7. ×75.

Fig. 9.—Healing stage at 5 months showing osteomyelitis. ×25.

rounded by a sheath of less compact bone which is rich in Haversian systems but contains only a few small marrow spaces. The site of the original defect is evidenced only by a small break in the continuity of the thin central compact core. The periosteum more nearly approximates in size that of the control area.

Radiographically the 4 months' stage shows complete filling in of the defect (Fig. 3*d*). Histologically there is a reduction in the gross size of the bone, although it is still approximately twice the size of the control side (Fig. 8). The exact area of defect can no longer be distinguished. The thin central core of compact bone is almost completely replaced by the less compact and

more marrow spaces are present. The periosteum is laying down an outer sheath of compact bone exactly similar in appearance and arrangement to the control side.

The fifth and sixth month stages are identical clinically, radiographically, and histologically. The typical picture of osteomyelitis with bone destruction and sequestration is seen on the x-rays (Fig. 3*e*). Histologic examination confirms the x-ray diagnosis (Fig. 9). However, the original defects can not be localized by x-ray or microscopic examination. It can be assumed that the defect is at least partially healed despite the presence of infection and the accompanying destruction of bone.

DISCUSSION

There exists a unanimity of opinion concerning the changes in the initial stages of the healing process following the fracture of any bone. Thus we can accept as factual the formation of a hematoma and the subsequent invasion by connective tissue. Therefore, the first experimental animal was not sacrificed until 6 weeks postoperatively at which time concrete evidence could be anticipated as to the type of repair in the operative site.

The radiographs taken immediately following sacrifice of the first four animals show a progressive obliteration of the radiolucent defect by a radiopaque area indistinguishable from the radiopacity of the normal surrounding bone (Fig. 3, *a-d*). It is logical to assume that the material filling in the defect represents calcified material giving the radiographic appearance of bone. The radiographs of the last two stages show a picture substantiating the clinical findings of multiple draining sinuses and leading to the proper diagnosis of osteomyelitis (Fig. 3*e*). It is worth noting that the original sites of the defects in the latter cannot be distinguished and that the entire malar process of the maxilla is involved in the osteomyelitic process.

The frontal section of the first four experimental stages when examined microscopically show the free edges bordering the defect to be in the sites of osteoblastic activity with the laying down of osteoid tissue (Figs. 5, 6, 7, and 8). This new osteoid quickly becomes calcified and assumes an architectural pattern cancellous in nature. The connective tissue bridging the gap is well differentiated. Simultaneously, with the attempt at bridging of the defect by the projection of new cancellous bone originating from the edges, there is a definite thickening of the periosteal membrane away from the operative site at the sides of the remaining normal bone. This increased periosteal activity results in the laying down of a less compact type of bone giving the entire area sufficient increase in thickness as to be distinguishable with the naked eye. The endosteum lining the Haversian systems adjacent to the defect also shows osteoblastic activity.

These three sites of accelerated bone production continue to show activity until the defect has been bridged; first by a junction of the thin cancellous projections of the edges, and second by a reinforced thickening of the entire malar process which extends across the gap and becomes indistinguishable from the initial thin bridging bone. Without entering into the controversy of the

etiology or significance of the cells which engage in new bone formation, it is correct to accept three distinct sites of accelerated bone production which contribute to the end result in this study. These three sites are: (1) the periosteum, (2) the endosteum lining the Haversian systems, and (3) the connective tissue callus filling the defect. In none of the sections is there any evidence of cartilage formation prior to the laying down of osteoid tissue. It is interesting to note that the osteoid tissue becomes calcified quickly and matures in nature as evidenced by the extreme thinness of this layer. By the end of the fourth stage (16 weeks) the site of the original defect is almost entirely obliterated, and the malar process in this area when examined microscopically appears to be composed of a thick central core of a cancellous type of bone with numerous marrow spaces. The periosteum is laying down a thin outer sheath of compact bone which is exactly the same pattern as seen in the normal control side with the exception of the greater over-all thickness of the malar process on the operated side.

The 20 and 24 week specimens gave the clinical and roentgenologic picture of osteomyelitis (Fig. 3*e*). This diagnosis is confirmed by the microscopic sections (Fig. 9). However, the original defect could not be distinguished roentgenographically or histologically in spite of the presence of infection.

It can be concluded, therefore, that in this study of operative interference with the continuity of the rat maxilla, in which the factors of infection, mobilization and apposition of the two edges are not present, the healing process results in bony union.

SUMMARY

The animals used in this study were 6 male albino rats. The zygomatic process was chosen as the operative site because it could be easily demonstrable radiographically. A dental burr was employed to cut a slot approximately halfway through the zygomatic process extending anteriorly from the free edge of its posterior border. Radiographs were taken before and after surgery and following sacrifice. The animals were sacrificed at intervals of 6 and 9 weeks and 3, 4, 5, and 6 months. The heads were fixed in 10 per cent formalin, decalcified in Custer's solution, imbedded in celloidin, sectioned serially in a frontal plane and stained with hematoxylin and eosin.

It can be concluded from the radiographic and histologic evidence that in this study of operative interference with the continuity of the rat maxilla, in which the factors of infection, mobilization, and apposition of the edges were eliminated, the healing process results in bony union. There is no evidence of a cartilaginous callus stage in the process of healing. The sites of new bone formation which repair the defect are: (1) the periosteum, (2) the endosteum, (3) the connective tissue callus. The end results in the presence of infection are bony union in spite of osteomyelitis.

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THE VASCULARITY OF THE INCISOR PULP OF THE MONKEY AND ITS ALTERATION BY TOOTH RETRACTION

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PREVIOUS experiments have shown that the pulps of the lower incisor teeth with large apical foramina might be destroyed by retractive force.[1] This retractive force was established by running rubber bands through small holes near the incisal borders and attaching their ends to a wire passed circumferentially around the mandible at the symphysis. The experiments indicated that the death of the pulp resulted from the lack of vascularity.

It has been questioned by some orthodontists if the pulp could be strangulated. Strang[2] was of the opinion that either sudden tension on a tooth might injure the vessels leaving the pulp chamber and cause serious venous congestion and the death of the pulp, or that a too powerful depressive force might shut off the arterial supply and thus produce a devitalization of the various elements in the pulp. Strangulation may, therefore, possibly occur, yet whether veins or arteries are most likely to be affected and exactly under what conditions they are affected remains to be demonstrated.

Information on these several points, i.e., what can be done and what should be avoided in altering the position of the teeth so as not to interfere too greatly with their vascularity would seem to be of sufficient importance to warrant further investigation.

Before describing the retractive effects, an account of the vascularity of the pulp must be presented since the literature contains so little about it.

PROCEDURE

Different monkeys (Rhesus) with permanent incisors in the pre-eruption stage, or erupted with large apical foramina, or erupted with small apical foramina were used in these investigations.

The monkeys were sacrified either with ether or gas and then decapitated. For the study of the vascular supply, the head was often perfused via the carotid with saline solution. However, perfusion usually did not increase the thoroughness of the injection. Various injection masses such as India ink, gelatin, latex, dichromate, and lead acetate were used. While the dichromate-lead acetate method of Williams[3] is ideal, the coloring is lost through decalcification. India ink, perfused through the head arteries, filled the pulp vessels sufficiently to well outline them after decalcification of the teeth. The blood vessels of the pulp in the lower central incisors were particularly difficult to fill.

This investigation was supported in part by a grant from the United States Public Health Service.

Received for publication, Sept. 7, 1951.

After injection the upper and lower jaws were removed, sawed into small pieces and fixed in Bouins' fluid. Following fixation the pieces containing the incisors were electrically decalcified, washed in water and embedded in paraffin. Cutting was performed on the sliding microtome. The sections of various thickness were stained in toto and mounted.

The procedure of retracting the teeth has been briefly reviewed in the introduction. Teeth with large apical foramina of different monkeys were investigated. Following retraction for various periods, similar procedure was followed in injecting and cutting the retracted incisors.

VASCULARITY OF THE PULP

The Pre-erupted Tooth.—The pre-erupted incisor is shown in Fig. 1. The pulp is dense throughout its extent, being slightly more dense apically. The blood vessels, without great change in size, course straight through the pulp. Between the root end of the tooth and the dense alveolar looser reticular tissue bone is found. Most of the veins converge toward the labial side of the tooth where they communicate with veins in the periodontal membrane. The epithelial root sheath extends beyond the apical growth of the dentin.

The Erupted Incisor With Large Apical Foramina.—The erupted incisor (Figs. 2 and 4) shows several changes from the pre-erupted tooth. The distal portion of the pulp becomes loose with the growth and elongation of the tooth while the apical area remains very dense. This apical region possesses potentialities of differentiating into pulp elements with further elongation of the root end. Blood vessels coursing through this dense portion are small in diameter and few in number. As they pass into the loose distal pulp they enlarge greatly. Arteries are more centrally located in the apical region and the veins are more often peripheral in position, draining usually around the labial side of the root end into the large veins of the periodontal membrane. A reticular soft tissue fills in between the alveolar bone and the dense apical pulp. This is very fragile and is very susceptible to shrinkage.

Fig. 3 shows cross sections through teeth such as that one shown in Fig. 2. The upper cross section is through the distal level (a) and shows more vessels and the looser pulp. The lower cross section is through the denser apical portion (b) of a tooth. The vessels of the apical pulp are smaller and particularly the veins are less numerous. Only three veins are seen in the apical cross section while many are apparent in the distal level.

A section of a maxillary incisor is shown in Fig. 4. The blood vessels of the incisors usually converge toward the labial side of the root end where the veins drain into those of the periodontal membrane. There is some drainage

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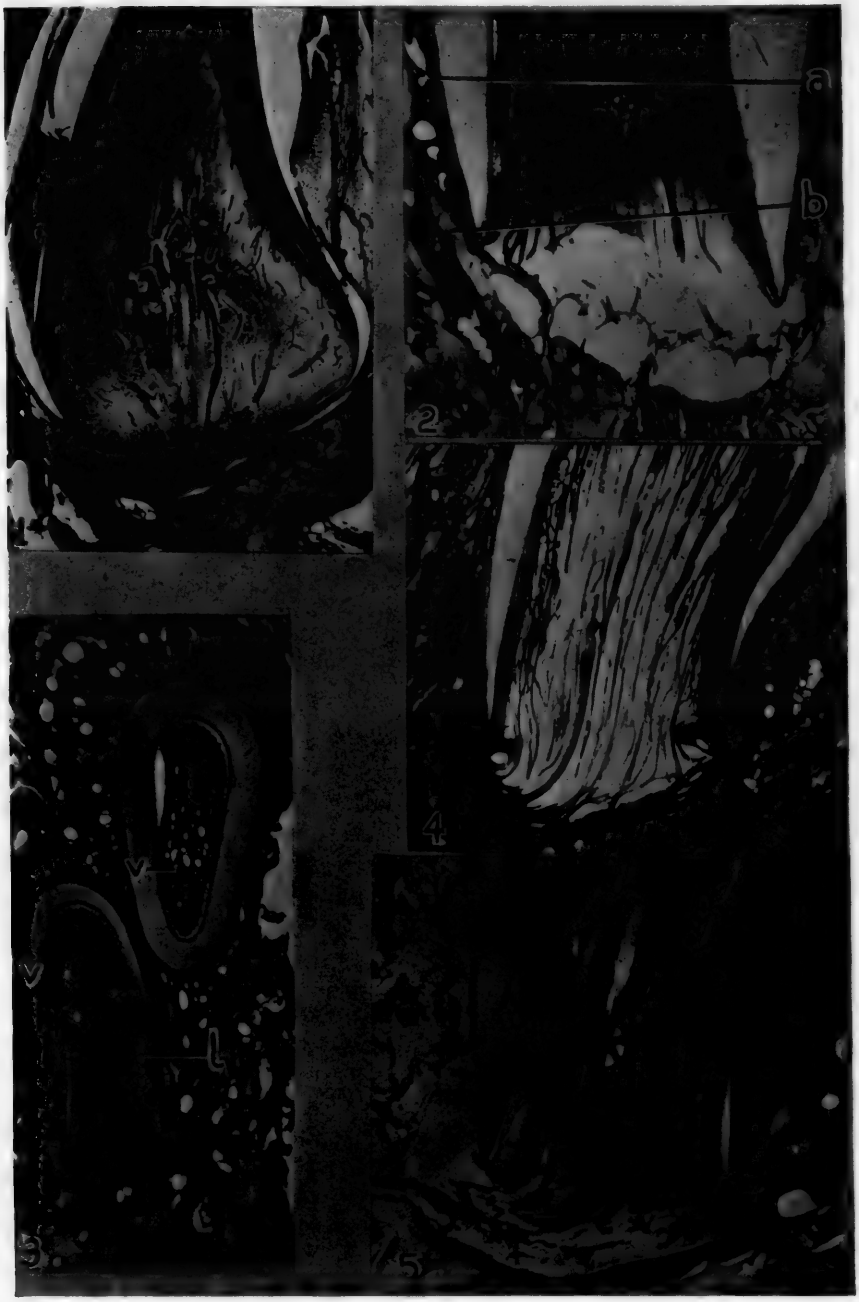
Fig. 1.—Normal pre-erupted tooth. Note the convergence of blood vessels toward the labial side of the tooth. ×12.

Fig. 2.—An erupted incisor, showing apical denser region of pulp which contains few blood vessels in comparison to the adjoining distal looser region with greater vascularity. ×25.

Fig. 3.—The right cross section is from the level *a* shown in Figure 2 and the left cross section is from level *b*. v = vein; l = lingual side. ×18.

Fig. 4.—Maxillary incisor showing convergence of vessels toward the labial side. ×12.

Fig. 5.—Shows a vein passing around the lingual side of the root end. ×25.



(For legends see opposite page.)

around the lingual side of the root end (Fig. 5) but little through the apical alveolar bone. The arterial supply, on the other hand, is greater through the apical alveolar bone into the pulp than it is around the margins of the root end. There is less loose tissue between the dense pulp and the alveolar bone in the maxillary incisors.

These studies give the impression that the pulp distally becomes loose in texture as the tooth elongates and erupts. Undoubtedly the looseness gives less support to the blood vessels, permitting an enlargement to a greater diameter than in those in the denser apical pulp. As the walls of the root end have thickened and elongated, the pulp at the apex has become compressed and changed morphologically. This growth and change have resulted in veins being pinched at the root end where they drain abruptly around margins of the root end into the large veins of the periodontal membrane.

The arteries course straight through the pulp, giving off branches which extend toward the dentine and continue as the capillaries. The capillaries form loops, many of which reach into the odontoblastic layer (Fig. 6). The blood is then returned to the wider dilated veins. Several instances have been found where the India ink has escaped from the capillary loop and penetrated into the dentinal tubules (Fig. 7).

Erupted Incisor With Small Apical Foramina.—Fig. 8 shows an older tooth with its small apical foramen. The large veins of the pulp are seen communicating around the margins of the root end with the large periodontal veins. The vessels enter the apex through several foramina which are separated by masses of atubular dentine. This dentine comes in close apposition with the underlying alveolar bone. The apical third of the pulp contains only a few large vessels which course directly through the pulp.

VASCULAR ALTERATION UPON RETRACTION

Erupted Tooth With Large Apical Foramen.—The retractive force of 250 Gm. used in these experiments served to push the root end of the tooth against the alveolar bone. This served to strangulate the veins at the root end and cause a stasis in the pulp. Fig. 9 shows a tooth which has been retracted for 72 hours. Small arteries at the root end and a few passing from the alveolar bone into the pulp contain India ink. Only two arteries contain any ink in the pulp. The veins in the pulp are congested with blood since the retraction prevented drainage by the major route, from the pulp into the periodontal vessels. Undoubtedly the retraction has altered the arterial supply of the pulp in two ways. Directly it has interfered with the artery and indirectly the venous congestion has provided a barrier to flow through the pulp. Shrinkage which is artificial has occurred in the soft tissue between the apex and the alveolar bone. Irregularity of the alveolar bone indicates that some resorption is occurring.

The tooth shown in Fig. 10 was retracted for 9 days. The root end contacted the bone and its margins have turned into the pulp. The pressure from the retractive force has caused a great congestion in the veins and prevented flow of blood into the tooth. Only the very apical portion of the pulp can possibly receive much vascularity. The odontoblasts have disappeared and the

Fig. 6.—Photograph showing the vascularity of the pulp. a = artery; c = capillaries; v = vein. ×75.

Fig. 7.—Photograph shows the penetration of India ink into the dentinal tubules. ×150.

Fig. 8.—Old tooth with vessels entering through several foramina. ×25.

Fig. 9.—Tooth retracted for 72 hours. Force at the beginning was 250 Gm. Note that the root end contacts the alveolar bone and that only two vessels appear in the pulp.

Fig. 10.—Another tooth which was retracted for 9 days. Areas of a few veins contained congested blood; i = ink; c = congested vessel.

pulp is necrotic. Resorption of the apical bone is occurring and a few osteoclasts are present. Fig. 11 shows the opposite retracted first incisor from the same monkey. It was retracted in the same way and for a similar period of 9 days. Despite the great pressure the pulp still has quite a vascularity and is surviving. Close observation of this tooth shows that the lingual side of the root end has been broken off indicating that the tooth was tipped lingually. The labial rim has not been forced so greatly and has not interfered with most of the vascularity. The opposite incisor (Fig. 10) was tipped more labially which resulted in greater interference with its vascularity, and death of its pulp resulted.

Fig. 12 shows the first and second incisors from another monkey. The first incisor, the tooth on the right, was retracted for 96 hours, while the second incisor was not altered. In the case of the retracted tooth the root end is against the alveolar bone, preventing much of the venous drainage. India ink has penetrated into the pulps of both teeth but much more is seen in the unaltered tooth, indicating that there has been interference with the vascularity of the pulp of the retracted tooth. At this magnification a difference may be noted in the contour of the alveolar bone around the apices of the teeth. The edge of the alveolus around the unaltered tooth is even while the irregularity of the margin of the alveolus around the retracted tooth indicates resorption. One can easily visualize, by looking at the figure, how retraction of the normal tooth would obliterate blood flow in many vessels to and from the pulp.

A higher magnification of the apical region of the same retracted incisor (Fig. 13) gives a better conception of the resorption that was taking place. The slight shrinkage in this instance was beneficial since it shows better the extent of resorption. In some instances there are indications of petechiae into the loose tissue at the apex of the tooth. A few osteoclasts were encountered in some areas of resorption but bone was disappearing in many places adjacent to connective tissue without osteoclasts.

In a thin section (Fig. 14) of a tooth which was retracted for only 48 hours the relations of the tissues and alveolar bone may be better seen at the apex. Extravasation has occurred. The pulp contains little blood.

The apex of another tooth from another monkey which had been retracted for 7 days is shown in Fig. 15. The root end is against the alveolar bone. The odontoblasts have degenerated and the pulp is necrotic. Tissues between the pulp and alveolar bone contain extravasated blood. Resorption of bone has taken place in many instances.

These various observations indicate that the venous drainage is more easily affected since a great stasis occurred yet some India ink was still able to pass into the arteries of the pulp. Considerable resistance to arterial flow

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Fig. 11.—Incisor which was tipped lingually. l.s. = lingual side. ×12.

Fig. 12.—The incisor on the right side was retracted for 96 hours while the adjoining incisor was unaltered. ×12.

Fig. 13.—Apical region of tooth retracted for 96 hours, showing that the alveolar bone is undergoing resorption. ×100.

Fig. 14.—Tooth retracted for 48 hours. Extravasation has occurred and the pulp is ischemic. ×100; a = alveolar bone; p = pulp, e = extravasation.

Fig. 15.—Root end of retracted tooth showing degeneration of pulp elements. ×100.

(For legends see opposite page.)

resulted at the root end and the pressure between the pulp and the alveolar bone provided resistance to arterial flow. The pressure of the pulp and tissues on the alveolar bone caused resorption of the bone. In several instances where the retraction force was continued for 10 to 20 days, enough bone was resorbed to relieve the resistance to blood flow and revascularization of the pulp began.

The vascularity of the pulp of teeth with small apical foramina was less often affected when retracted for similar periods and with similar force as that applied to teeth with large apical foramina. The root end did not contact the alveolar bone. There are several reasons why the retractive force was ineffective. In the first place the tooth is undoubtedly more securely attached by the periodontal membrane in the alveolus. The root end is thicker and is not altered as easily in any direction by the pressure. Furthermore, the wedge-shaped tooth more closely conforms to the alveolus and moves less easily in the apical direction.

DISCUSSION

The injections in the normal incisors indicate that most of the drainage is by veins which anastomose around the root end with the veins in the periodontal membrane. Most of these anastomoses are on the labial side of the tooth. Vascular supply is mainly by arteries coursing directly from the apical alveolar bone into the pulp. Boling[4] found 5 to 8 arterioles entering the roots of incisors of cats. Veins leaving the apex were more numerous, twelve being counted in the maxillary third incisor. Boling stated that the diameter of the veins in the pulp was much greater than those leaving the apex, indicating a sluggish flow within the pulp. In the monkey, the veins in the apical portion are smaller in diameter and they are also greatly reduced in number (Fig. 3). Veins leaving the apex are less numerous than the arteries. Lefkowitz and Waugh[5] have discussed the depression of teeth and described the changes in the periodontal membrane upon depression in the dog. Changes in the periodontal membrane after retraction of the teeth and after other injuries and their repair in the monkey will be made in a later report.

Bone resorption results from the pressure exerted at the apex of the tooth. This bone resorption relieves the resistance to blood flow. Much could be discussed about how and what is responsible for the bone resorption. It appears to occur in these sections either when the tissue is being pressed against the bone, when there is some extravasation around the bone or when there is an exudation of fluid around the bone. Pressure would seem to be the common factor in all cases.

Most important is the possibility of the orthodontist strangulating the vessels by his appliances. The orthodontist uses small forces compared with those used in these experiments. Second, his force is not exerted on the long axis of the tooth but is usually constructed for tipping the tooth. While tipping the tooth labially would interfere with most vessels, enough communicating vessels would probably still exist lingually for necessary vascularization. It, therefore, is possible, yet not very probable that any appliance of the orthodontist would have such a force or direction as to strangulate the vessels to the extent of

causing a necrosis of the pulp. In many instances, as shown by our previous experiments, the necrotic pulp would be invaded and repopulated by growth of tissue from the apical region.

CONCLUSIONS

Most of the venous drainage of the pulps of the incisor teeth in the monkey is via veins which are continuous around the rim of the root end with the veins of the periodontal membrane. The continuations are more frequent on the labial side.

Most of the vascular supply appears to be by arteries which course directly from the alveolar bone into the apex of the teeth.

Retraction exerted on the long axis of the teeth with large apical foramina may push them with sufficient force to strangulate the veins and interfere with the arterial supply of the pulp. The vessels of old teeth with small apical foramina are seldom strangulated, apparently because of their form and more secure periodontal anchorage.

The retractive force causes a resorption of bone in the apical region of the teeth. This process relieved the resistance to the blood flow.

Although there is more danger of altering the blood vessels by tipping the tooth labially, there is little evidence that the retractive force applied by orthodontists would be sufficient to strangulate the vascularity of the pulp.

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MORPHOLOGIC RELATIONSHIPS BETWEEN ENAMEL AND DENTIN SURFACES OF LOWER FIRST MOLAR TEETH

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IT IS well known that the establishment of the dentino-enamel junction by the juxta-alignment of the inner enamel epithelium and the subjacent mesenchymal cells provides the anlage for the future morphologic characteristics of the completed tooth. How closely the completed enamel crown, particularly its occlusal surface, mirrors the dentino-enamel junction (which will now be regarded as the surface of the dentin "crown") is not known. Nor has the metrical cusp-to-cusp correspondence of enamel to dentin surfaces been studied. The latter obviously must be a function of growth rate. A uniformity of topical and metrical relationships between the enamel and dentin surfaces bespeaks a uniform pattern and rate of enamel deposition in the growing tooth. Thus far it has been more or less tacitly assumed that there is complete correspondence between the two surfaces. Schour and Massler[1] stated that "the dention-enamel junction or blueprint pattern of the tooth form is characterized by definite high points upon its surface which correspond in number and position to the cusps (in the posterior teeth) or lobes (in the anterior teeth) of the completed tooth."

In order to ascertain whether or not complete topical and metrical correspondences exist between the enamel and dentin surfaces, a study was made of 50 lower first molars. If such correspondences did in fact exist then it could be assumed that the daily rate of formation of enamel proceeds uniformly throughout the enamel crown.[2] If the enamel surface did not show such correspondence with the dentino-enamel junction then there must be a differential growth rate or pattern in the formation or apposition (or both) of enamel in the growing tooth, In the latter event a histologic study of the course of the enamel rods in various portions of the crown might provide some answers.

If consistent correlations in topical arrangements could be demonstrated between enamel and dentin surfaces, then a valuable tool for the study of cuspal arrangements on molars showing varying degrees of attrition would be available. For example, to study the crown patterns of worn fossilized primate teeth, it would be necessary only to remove the remaining enamel and study the basic dentino-enamel surface, reconstructing the enamel arrangement in terms of established correlations. Indeed, it was largely with this purpose in mind that the present study was inaugurated.

The present study is one of a series of investigations of the genetic factors involved in the human dentition that are being inaugurated at the Laboratory of Physical Anthropology, University of Arizona. They are aided by a Fellowship Grant from the John Simon Guggenheim Memorial Foundation, awarded for 1951-1952.

Received for publication, Nov. 5, 1951.

METHOD AND MATERIALS

Fifty lower first permanent molars were employed in this study. They were extracted from the mandibles of skeletons recovered from the prehistoric Arizona sites of Tuzigoot and Kinishba, dating roughly around A.D. 1200. All of the first molar teeth were unerupted but with root formation in the beginning stages; thus there was no wear on the enamel of any of the teeth. Half the teeth were lower left molars, half lower right. Of the total number of teeth there were 15 pairs, each representing lefts and rights from the same jaw.

The maximum crown breadth and length of each tooth was measured first. Impressions were then taken of each molar crown and casts made from these for later checks and comparisons. On each molar the apex of each cusp (except the hypoconulid) was marked with a lead pencil held so that the conical margin of the pencil was tangential to a plane established between the apices of any two cusps. The tooth was then inverted and the cuspal apices were impressed upon paper resting on a hard level surface so that the paper bore pencil impressions of the cuspal apices. It was necessary to rock the tooth slightly to make all 4 impressions since the apices were not all on the same horizontal plane.

The following measurements were made directly on the paper, using a Helios caliper with Vernier scale:

1. Diameter between protoconid and metaconid apices.
2. Diameter between hypoconid and entaconid apices.
3. Diameter between protoconid and hypoconid apices.
4. Diameter between metaconid and entaconid apices.
5. Diameter between protoconid and entaconid apices.

The apices of the enamel cusps were plotted on graph paper, using a 10:1 scale.

The enamel portion of each molar crown was then removed by placing the tooth in a beaker containing a 9 per cent solution of HCl at about 70° F. The tooth rested on a ni-chrome wire gauze to facilitate removal without handling. Each tooth was placed with the occlusal surface up, and inferiorly exposed pulp cavities were covered with hot dental baseplate wax to prevent action of the solution on dentin. Immersion time varied from 9 to 24 minutes, the tooth being inspected frequently to note stage of enamel removal. In all cases the enamel was completely removed without involvement of the dentin, although some of the main intercusp fissures retained their enamel covering. The teeth were then rinsed with water and handled carefully so as to prevent chipping of the dentinal cusps.

Interapical diameters on the dentin cusps were measured directly, since the apices are sharply defined. The same measurements were made as on the enamel cuspal apices. The dentin apical points were then plotted on the same 10:1 scale within the outline of the enamel apical perimeter for the same tooth. The points of the enamel and dentin apices of the protoconid were made to coincide, as were the protoconid-hypoconid diameters.

Analysis of the metric characteristics of the cuspal apices of both enamel and dentin surfaces and comparison of absolute and relative dimensions and angles proceeded on the basis of the graphs.

RESULTS

The angle formed at the entaconid apex by the lines connecting the metaconid, entaconid, and hypoconid apices, proved to be significantly the smaller of the 4 apical angles on both dentin and enamel surfaces. The mean entaconid angle on the enamel was 74.6°, and on the dentin, 70.9°. The largest mean angle, though not significantly so, was that formed at the hypoconid apex on both enamel and dentin. The mean metaconid angle was only slightly smaller on both surfaces than the hypoconid angle, and the protoconid angle showed a mean that was significantly lower than the metaconid and hypoconid means and significantly higher than the entaconid means for both enamel and dentin surfaces. (See Table I.) On both the dentino-enamel junction and on the completed enamel surface, therefore, the apex of the entaconid projects sharply distolingually from the rest of the body of the tooth.

TABLE I

MEANS AND STANDARD DEVIATIONS OF THE ANGLES FORMED BY THE CUSPAL APICES ON THE ENAMEL AND DENTIN SURFACES

APICAL ANGLE	MEAN	S.D.	V.
Enamel			
Protoconid	92.8 ± 1.14	±12.03	±13.0
Metaconid	94.1 ± 1.07	±11.28	±12.0
Entaconid	74.6 ± .95	±10.04	±13.4
Hypoconid	95.1 ± 1.09	±11.43	±12.0
Dentin			
Protoconid	92.0 ± 1.33	±12.76	±13.9
Metaconid	98.3 ± 1.22	±11.79	±12.0
Entaconid	70.9 ± 1.04	± 9.98	±14.1
Hypoconid	98.4 ± 1.23	±11.89	±12.1

The mean difference between the entaconid angles on the enamel and dentin surfaces of the same tooth was 6°. Of 42 teeth measured, 26 showed increases of the enamel angles over the dentin angles at the entaconid, 13 showed decreases, and 3 showed no change. For the protoconid angles the mean difference was 9°, with 26 showing increases of the enamel angles over the dentin angles (mean increase of 9.9°). Thirteen teeth showed mean decreases in the enamel angles of 7.3°. The hypoconid shows a greater tendency for a decrease in the enamel angle as compared with the dentin angle. Twenty-three teeth showed a mean decrease of 9°, while 17 teeth showed an average increase of 5.6°. The total mean change in enamel hypoconid angle compared with the dentin hypoconid angle was 7.5°. With regard to the metaconid angle, there is an even greater tendency toward decrease of the enamel angle, 25 teeth having a mean decrease of 9.2°. Only 14 teeth showed enamel angle increases, with a mean of 13.3°. The total mean change for the metaconid angle was 10.7°.

To sum up, there is less change (hence greater correspondence) in the topical relationship of the entaconid apex to the apices of its 2 adjacent cusps, comparing its enamel and dentinal components, than of any other cuspal apex. The enamel entaconid and protoconid angles are twice as likely to show increases over the dentin entaconid angles as to show decreases. The enamel metaconid and hypoconid angles, on the other hand, are twice as likely to show

decreases as compared with their dentinal counterparts. The greatest differentiation in angulation between enamel and dentin apices occurs in the metaconid (10.7°), next in the protoconid (9.0°), and third in the hypoconid (7.5°). With respect to the relative position of the cuspal apices, then, the correspondence between enamel surface and dentino-enamel junction is greatest at the distolingual portion of the tooth.

The 4 straight lines connecting the cuspal apices of both enamel and dentin surfaces form what might be called apical perimeters. Each enamel perimeter is larger than its respective dentin perimeter. The mean enamel perimeter is 21.44 mm.; the mean dentin perimeter is 18.86 mm. The very low probable errors of both means indicate almost an impossibility ($\frac{x}{P.E} = 15.8$) that the dentin perimeter would ever exceed its enamel perimeter. Both absolutely and relatively the deviation from the mean perimeter for the enamel was less than for the dentin.

Table II reveals certain interesting correspondences between the enamel and dentin apical dimensions. By computing for each tooth the relationship of a single diameter, the hypoconid-entaconid diameter for example, to the apical perimeter of that tooth we arrive at an index ($\frac{\text{bi-apical diam.} \times 100}{\text{apical perimeter}}$) by which we may compare relative distances between the enamel and the dentin cuspal apices. The means and standard deviations for these indices are presented in Table II. For both the enamel and dentin cusps the hypoconid-entaconid apical diameter is significantly the larger, while the protoconid-hypoconid diameter is significantly the smaller. Moreover, the correspondence between enamel and dentin surfaces in the respective values of all 4 bi-apical indices is strikingly high, the greatest difference—.82—occurring for the protoconid-metaconid diameter.

TABLE II

MEANS AND STANDARD DEVIATIONS OF ENAMEL AND DENTIN BI-APICAL DIMENSIONS, EXPRESSED IN TERMS OF THE INDEX: $\frac{\text{BI-APICAL DIAMETER} \times 100}{\text{APICAL PERIMETER}}$

BI-APICAL DIMENSION	RANGE	MEAN		V.
Enamel				
Hypoconid-entaconid	25.4-30.9	28.7 ± .12	±1.27	±4.43
Protoconid-metaconid	20.7-29.6	24.1 ± .17	±1.83	±7.6
Protoconid-hypoconid	17.3-24.7	20.9 ± .16	±1.70	±8.14
Metaconid-entaconid	20.6-29.3	26.2 ± .18	±1.94	±7.42
Dentin				
Hypoconid-entaconid	24.0-31.4	28.3 ± .14	±1.45	±5.13
Protoconid-metaconid	18.8-28.6	23.3 ± .19	±1.98	±8.5
Protoconid-hypoconid	17.0-24.8	21.6 ± .15	±1.58	±7.32
Metaconid-entaconid	19.6-31.6	26.6 ± .23	±2.40	±9.0

When the apical dimensions are compared directly, enamel to dentin, significant differences are found. (Table III.) There is an absolute increase in each enamel apical dimension over that of its respective dentin analogue. This holds not only for the means but for each pair of dimensions on each tooth. The greatest mean increase is shown in the entaconid-hypoconid diameter. This means that the enamel entaconid apex and the enamel hypoconid apex

TABLE III

MEAN ABSOLUTE DIFFERENCES BETWEEN THE RESPECTIVE ENAMEL AND DENTIN BI-APICAL DIAMETERS

BI-APICAL DIMENSION	ENAMEL-DENTIN DIFFERENCE IN MILLIMETERS			
	RANGE	MEAN	S.D.	V.
Protoconid-entaconid	.2-1.9	.89 ± .006	±.037	±4.2
Protoconid-hypoconid	.1-1.2	.40 ± .003	±.025	±6.2
Metaconid-entaconid	.2-1.6	.60 ± .004	±.029	±4.8
Protoconid-metaconid	.1-2.3	.73 ± .006	±.045	±6.2
Entaconid-hypoconid	.2-2.2	.81 ± .006	±.042	±5.2

lie considerably lingual and buccal, respectively, to their dentin analogues. The next greatest increase is found in the protoconid-metaconid diameter. It becames apparent, therefore, that enamel growth, whatever its mechanics, proceeds in such a manner that the completed enamel apices are displaced linguo-buccally more than mesiodistally relative to their dentin analogues. However, the ultimate position of the enamel cuspal apex relative to its dentin analogue appears to be a vector, the result of differential growth forces proceeding in 2 directions. For the entaconid apex, for example, there appears to be a differential lingual and distal enamel growth. A final explanation of these data must await more detailed histological examination.

It was assumed by the author that right and left lower first molars from the same jaw would show greater similarities in the absolute and relative increases of enamel bi-apical diameters over the corresponding dentin diameters than would teeth drawn from different jaws. Such was not uniformly the case, as seen in Table IV. The mean difference in enamel protoconid-metaconid apical diameters between left and right molars from the same jaw was .44 mm., in the same dentin diameter the mean difference was .45 mm. For the metaconid-entaconid diameter the mean difference in enamel was .52 mm., in the dentin, .39 mm.; for the entaconid-hypoconid diameter, .55 mm. in the enamel, .65 mm., in the dentin; for the hypoconid-protoconid diameter, .53 mm. in the enamel, .46 mm. in the dentin; and for the diagonal diameter, the protoconid-entaconid apical distance, the enamel difference was 0.5 mm. and the dentin 0.6 mm. When these differences are compared with the mean differences between left and right molars taken from different jaws at random, it is found that for the entaconid-hypoconid and hypoconid-protoconid diameters on both enamel and dentin surfaces there is greater similarity between molars

TABLE IV

MEAN DIFFERENCES IN ENAMEL AND DENTIN BI-APICAL DIAMETERS BETWEEN PAIRED AND UNPAIRED LEFT AND RIGHT LOWER FIRST MOLARS

BI-APICAL DIAMETERS	ENAMEL DIFFERENCES IN MILLIMETERS		DENTIN DIFFERENCES IN MILLIMETERS	
	PAIRED	UNPAIRED	PAIRED	UNPAIRED
Protoconid-metaconid	.44	.68	.45	.63
Metaconid-entaconid	.52	.59	.39	.71
Entaconid-hypoconid	.55	.43	.65	.52
Hypoconid-protoconid	.53	.41	.46	.34
Protoconid-entaconid	.50	.71	.59	.69

from different jaws! The other 3 diameters show greater similarity in the paired teeth. While the sample from which these data are derived is too small to warrant any statistical reliance, it does seem to indicate that for the lower first molars at least, growth rate as measured in terms of dentino-enamel relationships is not bilaterally symmetrical, and suggests that the hereditary determinants of enamel growth may have unilateral limitations.

These suggestions are further borne out by comparing pairs of molars from the same jaw with pairs of molars from different jaws with respect to the percentage increase of the enamel over the dentin bi-apical diameter. Computing the index, $\frac{\text{(enamel bi-apical diameter)} - \text{(dentin bi-apical diameter)} \times 100}{\text{(dentin bi-apical diameter)}}$, for each tooth, the difference in percentage increase for each pair of molars (from same and different jaws) was obtained. Then the mean difference in percentage increase for the 15 paired and for the 15 unpaired molars was computed for each bi-apical diameter. (Table V.) Again, while we might expect a significantly smaller difference for molars from the same jaw, such is not the case. Apparently the relative increase in enamel growth as reflected in bi-apical diameters is the result of unilaterally operative growth phenomena. In two bi-apical diameters the mean difference is smaller in unpaired molars. The small size of the sample again precludes positive conclusions but strongly suggests unilateral growth factors.

TABLE V

MEAN DIFFERENCES IN THE RELATIVE ENAMEL INCREASE FOR PAIRED AND UNPAIRED MOLARS WITH RESPECT TO BI-APICAL DIAMETERS

BI-APICAL DIAMETER	MEAN DIFFERENCE IN ENAMEL INCREASE IN PER CENT	
	PAIRED TEETH	UNPAIRED TEETH
Metaconid-entaconid	5.30	4.53
Protoconid-metaconid	11.40	8.16
Entaconid-hypoconid	6.99	8.67
Hypoconid-protoconid	3.67	3.90

On the basis of the same index corroborative evidence for the greater linguo-buccal displacement of the cuspal apices is brought to light. Computing the mean relative increase of enamel for each bi-apical diameter of the 30 teeth of the paired series, the following figures are obtained:

BI-APICAL DIAMETER	MEAN RELATIVE INCREASE OF ENAMEL (PER CENT)
Hypoconid-protoconid	8.0
Metaconid-entaconid	11.2
Entaconid-hypoconid	16.5
Protoconid-metaconid	15.6

Both linguo-buccal bi-apical diameters show significantly greater enamel increases over the anteroposterior diameters. The hypoconid-protoconid diameter shows the least relative enamel increase.

Some further insight into the nature of the differences between enamel and dentin crowns may be gained by an examination of the maximum widths and lengths of the lower first molar teeth. The mean maximum dimensions and

their probable errors and deviations are presented in Table V. The maximum width and length of the dentin crown are equal. These proportions are altered, however, on the enamel crown, where the mesiodistal diameter becomes relatively greater. For each of the 50 molar teeth studied the enamel length was greater than the enamel width, regardless of the fact that for some the dentin length was less than the dentin width. The difference between the mean enamel length and width is statistically significant, since the difference between the 2 means is more than eleven times the probable error of that difference. Again, though there appear to be differential growth forces operative, the growth mechanism has not yet been examined. That the hereditary factor is important

TABLE VI

MAXIMUM WIDTH AND LENGTH OF ENAMEL AND DENTIN CROWNS OF 50 LOWER FIRST MOLAR TEETH. (IN MILLIMETERS)

DIMENSION	RANGE	MEAN	S.D.	V.
Enamel				
Max. Length	10.2-12.9	11.1 ± .05	±.5	±4.9
Max. Width	9.2-11.9	10.3 ± .05	±.5	±5.1
Dentin				
Max. Length	7.9-10.3	8.9 ± .05	±.6	±6.4
Max. Width	7.6-10.3	8.9 ± .06	±.6	±6.9

seems to be suggested by the consistency of the greater enamel increase of the cusp dimensions in the mesiodistal plane. It should be pointed out that while the maximum enamel crown growth is in the mesiodistal axis, the maximum enamel apical displacement (relative to the dentin apical positions) is in the linguo-buccal axis.

A final consideration is the absolute and relative differences between the maximum enamel and dentin widths (and lengths) for each molar tooth. The statistical data are presented in Table VII. There is little absolute difference between maximum widths and lengths in this characteristic, the mean difference being only 0.07 mm. However, in terms of the relative difference—expressed as the ratio of enamel-dentin difference in length (or width) to absolute dentin length (or width)—we find that the mean relative increase of enamel length is 26 per cent as compared with the mean relative increase of enamel width of 16 per cent. In other words, relative to the dentin crown the enamel grows at a faster rate (or longer) in the mesiodistal axis than in the linguo-buccal axis, the same conclusion reached previously on slightly different grounds.

TABLE VII

ABSOLUTE AND RELATIVE DIFFERENCES BETWEEN ENAMEL AND DENTIN CROWNS OF 48 LOWER FIRST MOLAR TEETH IN MAXIMUM WIDTH AND LENGTH

DIMENSION	RANGE		MEAN		S.D.	
	(MM.)	(%)	(MM.)	(%)	(MM.)	(%)
Length	.6-3.8	15.7-41.7	2.4 ± .06	26.1 ± .7	±.6	±7.4
Width	.2-3.1	6.5-37.4	1.5 ± .05	16.2 ± .6	±.5	±6.7

DISCUSSION

An interpretation of the results obtained in this study must be referred constantly to the limitations imposed by the size and character of the sample as well as to the restricted scope of the investigation. Inevitably, in this type of study, many more problems are posed than are solved. Since the teeth involved are those of a prehistoric Southwestern Indian group it is certainly questionable whether the resultant data are applicable to all human lower first molar teeth. Further sampling is required for confirmation.

A gross morphologic study of this nature can not be truly definitive without the histologic analysis necessary to reveal the underlying structural characteristics responsible for the observed external features. Both types of study cannot be undertaken on the same tooth. Hence a much larger sample must be worked to confirm the results herein set forth. With such confirmation, histologic examination of other teeth drawn from the same population can proceed with some assurance that the results obtained will be applicable to the gross morphologic findings.

It seems apparent, however, from the present results that there is no precise metrical or topical correspondence between the cuspal apices of the enamel crown and their dentin homologues. This being so, there is no possibility of reconstructing the topography of the enamel occlusal surface from the pattern presented by the dentino-enamel junction with either metrical or topical precision. While it remains true that there is a certain nonmetrical correspondence (the number of cusps and the crown pattern in general) between enamel and dentin occlusal surfaces, there are now revealed certain patterns wherein the metrical relationships between enamel and dentin surfaces and crown features differ.

It is presumed by the author that genetic factors are operative to produce such constant differences, among which are to be observed the predictable changes in enamel crown shape and the greater linguo-buccal displacement of the enamel cuspal apices relative to their dentin homologues. These genetic factors, furthermore, appear to be unilaterally independent, since the observed traits are differentiated to the same degree between left and right molars taken from the same jaw as between lefts and rights from different jaws. Whether a modified genetic field concept is applicable to these growth phenomena can only be determined by extension of this type of study to unilateral molar rows.

SUMMARY AND CONCLUSIONS

Measurements were taken of enamel and dentin cuspal bi-apical diameters on 50 lower first molar teeth taken from skeletons of prehistoric Arizona Indians. Maximum widths and lengths on both enamel and dentin crowns were also measured. The following results were obtained:

1. On both enamel and dentin surfaces the angle at the entaconid apex was significantly the smaller of the 4 apical angles (hypoconulid was not considered in this study).

2. There was no consistent nor constant increase or decrease in the enamel apical angles as compared with the dentin apical angles.

3. There was greater correlation in angulation between enamel and dentin apices at the entaconid.

4. The enamel apical perimeter was consistently larger than its corresponding dentin perimeter, and both relatively and absolutely was less deviant from the mean.

5. For both enamel and dentin surfaces, the hypoconid-entaconid apical diameter is significantly the larger and the protoconid-hypoconid diameter significantly the smaller.

6. The protoconid-metaconid and hypoconid-entaconid bi-apical diameters show the greatest increase, both absolute and relative, in enamel over dentin. The enamel apices thus show more linguo-buccal than mesiodistal displacement relative to their dentin homologues.

7. The various enamel and dentin bi-apical relationships are not bilaterally symmetrical. There are as large differences between paired as between unpaired lower first molars.

8. Means of the maximum crown width and length for the dentin were of the same magnitude, but for the enamel the mean maximum length was significantly greater.

It is suggested that the metrical and topical differences between the enamel and dentin surfaces are the expression of genetic enamel growth factors which apparently operate unilaterally. Some insight into the nature of these factors might be provided by histologic examination.

The author is indebted to Dr. A. A. Dahlberg, Dental Surgeon (R) United States Public Health Service, for proposing the problem of enamel-dentin relationship and suggesting specifically the techniques outlined herein; also to Delbert Fleck, graduate student and dental technician, who removed the enamel and aided the author in the statistical analyses and in checking all measurements.

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THE CORRECTIVE EFFECT OF PARATHYROID HORMONE ON GENETIC ANOMALIES IN THE DENTITION AND THE TIBIA OF THE *ia* RAT

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THE *ia* mutation in the albino rat is characterized by a retardation of resorption of bone tissue in the entire skeleton (hereditary hypo-osteoclasis). As a consequence, the developing and growing bones show generalized osteosclerosis. In the jaws, the lack of physiologic resorption of the alveolar process leads to the distortion and ankylosis of developing teeth. A previous study[1] showed that the proliferation and differentiation of the odontogenic epithelium is normal. However, as the tooth germs continue to grow, but become increasingly impinged upon by nonresorbing alveolar process, the odontogenic epithelium, dentin, enamel, and bone accumulate in odontome-like formations and the teeth become ankylosed and fail to erupt. The long bones are nearly normal in their shape and rate of growth, but show osteosclerosis which is especially marked in the metaphyseal and epiphyseal regions.[2]

Since it was established that the primary cause of the abnormalities of the *ia* skeleton and dentition was the retardation of physiologic resorption, it was decided to investigate the effect of the administration of parathyroid hormone upon the *ia* skeleton and alveolar bone. The results of this study indicate that the anomalies of the *ia* mutation can be corrected by timely administration of parathormone.

METHOD AND MATERIAL

This study is based on the roentgenologic and histologic analysis of the teeth with their supporting structures and the tibias of 3 groups of *ia* rats (Table I).

Group A consisted of 17 untreated *ia* rats, ranging in age from 3 to 42 days.

Group B consisted of eight *ia* rats, ranging in age from 8 to 15 days, which received 7 daily injections of 10 to 25 international units (I.U.) of parathormone.

Group C consisted of 12 *ia* rats, 37 to 45 days of age, which received 33 to 39 daily injections of 10 to 25 international units (I.U.) of parathormone.

The administration of the hormone in Groups B and C was started between the second and ninth day after birth.

Bisected heads and the tibias were radiographed, decalcified, embedded in celloidin, sectioned serially and stained with hematoxylin and eosin. Some selected sections were stained with Mallory's connective tissue stain and Gomori's silver impregnation method.

This investigation was supported in part by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service.

Presented at Twenty-Eighth Annual Meeting of the International Association for Dental Research, French Lick, Indiana Harbor, March 24-26, 1950. (J. D. Res. 29: 673, 1950.)

Received for publication Dec. 10, 1951.

TEETH FINDINGS

1. *Roentgenologic Observations.*—

Group A: In the untreated *ia* rats none of the teeth had erupted. In the place of the incisors and molars, irregular radiopaque areas, indicating pseudo-odontomes, could be seen (Fig. 1).

Group B: The animals of Group B which received 7 injections of the parathyroid hormone over a period of 7 days did not show any radiographically significant differences from the untreated *ia* rats.

Group C: In this group the *ia* rats received 33 to 39 injections of parathormone. They showed either the absence or a reduction in the size of the pseudo-odontomes. Although the odontome-like formation both in the upper and lower jaws showed response to treatment, the most striking results were obtained in the regions of the upper incisors and molars.

The incisors in one of the treated *ia* rats of Group C had erupted and the teeth showed normal periodontal membrane width and absence of the odontomes (Compare Figs. 1 and 2). In all other animals of Group C the incisors failed to erupt. However, a radiolucent area, absent in the untreated rats, could be seen between the distorted upper incisors and the alveolar bone (Compare Figs. 1 and 3). In addition, the odontomes seemed less irregular and less radiopaque than the controls.

The molars of the treated *ia* rats of Group C showed a partial or complete absence of odontomes. Roots had partially or completely formed. A periodontal space around the roots could be seen. With the exception of 3 animals, which received the parathyroid hormone beginning at 9 days of age, the upper second and third molars had erupted in every case (Figs. 2 and 3). Some of the lower second and third molars had erupted in 3 animals of Group B which started receiving the parathyroid hormone at 4 or 5 days of age. In this group the first molars, which in the untreated *ia* rats are most severely affected, showed a partial formation of their roots. They had erupted in three animals in which the treatment had begun at 4 or 5 days of age (Fig. 2).

2. *Histologic Observations.*—Since the histologic as well as the roentgenologic study showed that the effect of parathyroid hormone was more striking in the upper than in the lower teeth, only the changes in the upper incisors and molars will be presented.

Group B: Though the roentgenologic findings were negative, the histologic study of the teeth of this group showed that the *ia* anomalies were far less severe than in Group A (untreated *ia* rats).

Some animals were treated from 3 to 10 days of age. The corrective effect of the hormone was more marked in the rats which started receiving the hormone at 3 days of age than in the rats in which administration was started at 9 days. At 10 days of age the incisor of the untreated *ia* rats consisted of two portions: The incisal tip which was normally developed; and the basal portion which showed an irregular arrangement of enamel, dentin, alveolar bone and pulp

(Fig. 6). The odontogenic epithelium was folded and had proliferated into the bone marrow spaces and had induced the formation of dentin and enamel (pseudo-odontome).

In the incisor of the *ia* rats treated from 3 to 10 days of age, a third portion could be distinguished at the basal third of the incisor (Fig. 7). Here, the odontogenic epithelium, dentin, and enamel had developed normally. Unlike the untreated rats, the epithelium of the germinative end was unbroken. Whereas in the untreated animals, bone trabeculae of the crypt encroached upon the distorted portion of the incisor, in the treated animal a periodontal membrane intervened between the tooth germ and the surrounding bone. The side of the bone trabeculae facing the incisor showed resorption by osteoclasts.

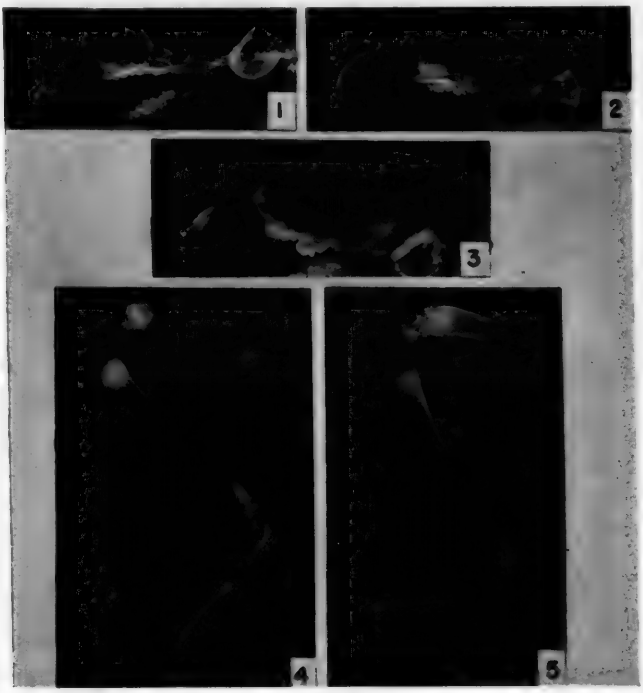

Fig. 1.—Roentgenogram of bisected head of untreated *ia* rat 42 days of age. Note odontome in region of upper incisors. None of molars have erupted.

Fig. 2.—Roentgenogram of bisected head of treated *ia* rat 37 days of age. Injections of parathormone were started at 5 days and animal received total dosage of 690 I. U. Note that incisor and all molars have erupted.

Fig. 3.—Roentgenogram of bisected head of treated *ia* rat 43 days of age. Note reduction in irregularity of odontome in upper incisor region. Anteroposterior length of distorted upper incisor is longer than in control (Fig. 1), and around upper incisor radiolucent area is seen. Upper second and third molars have erupted.

Figs. 4 and 5.—Roentgenograms of posterior extremities of an untreated (Fig. 4) *ia* and a treated *ia* littermate (Fig. 5) 42 days of age. The administration of parathormone in latter animal was started at 9 days and it received total dosage of 655 I. U. Note the radiopaque areas in the metaphyses of the untreated tibia and femur. In treated littermate long bones appear almost normal.

Other animals were treated from 9 to 15 days of age. In the animals of Group B which started receiving parathyroid hormone at 9 days of age, and which were sacrificed at 15 days, the changes were less striking. As compared

with the untreated rats, the incisor teeth of these animals were less distorted and the odontogenic epithelium showed signs of normal organization (Compare Figs. 8 and 9). However, the periodontal membrane and the bony crypt were not as well organized as in the animals which started receiving the parathyroid hormone at 3 days of age. Bone resorption was in progress.

The first and second molars in this group were less distorted than those of untreated rats. The third molar tooth germs were not distorted, since even in the untreated *ia* rats the distortion of the odontogenic epithelium of the third molar does not begin till about 20 days of age.

Group C: The upper incisors of one of the rats which started receiving injections of parathyroid hormone at 5 days of age had erupted (Compare Figs. 10 and 11). Except for their shape these teeth were normal in all respects. Their curvature was flatter than normal, and the incisal tips were distorted. The distorted incisal tips were apparently the portions of the teeth which developed before the administration of the hormone was begun. The pattern of apposition and resorption of the alveolar bone was normal. On the concave side the premaxillary part of the alveolar bone showed bone apposition. The part of the alveolus which was formed by the maxilla and which faced the pulpal opening showed bone resorption. On the convex side the premaxillary part of the alveolus showed bone resorption and the maxillary part showed apposition of bone. The periodontal membrane was well developed. The basal end of the incisor had grown into the maxilla, but not to the same degree as would be expected in a normal incisor of the same age.

In other animals of this group the incisors did not erupt. However, the distortion of the incisors was less and the anteroposterior length of the incisors was greater than in the untreated *ia* rats.

In two animals of Group C, all the upper molars had fully erupted and their roots were completely formed (Compare Figs. 12 and 13). There was a normal periodontal membrane and the surface of the alveolar bone facing the molars showed the normal pattern of apposition and resorption.

In 7 animals of Group C, which started receiving the parathormone at 4 or 8 days of age, only the second and third molars had erupted (Compare Figs. 12 and 14). The root formation of these teeth was either completed or in the process of completion. Although the roots were sometimes short, the width and structure of the periodontal membrane were normal (Fig. 14). The unerupted first molars in these animals showed much less distortion than in the

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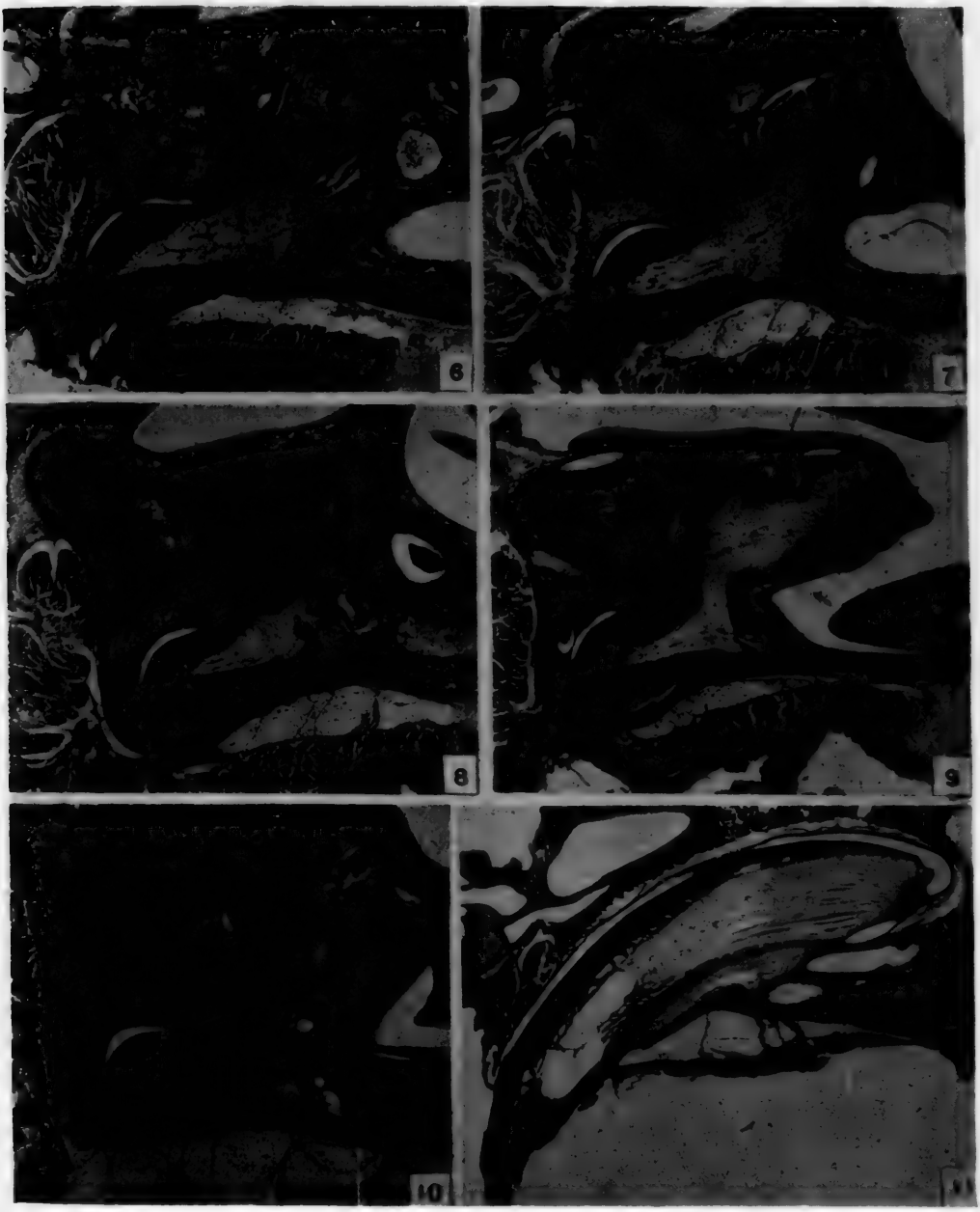
Fig. 6.—Untreated *ia* rat 10 days of age. Note that the distorted upper incisor has essentially two parts: anterior undistorted and posterior part which is completely disorganized. ×21.

Fig. 7.—Treated *ia* rat 10 days of age. Note that upper incisor has 3 parts. Anterior undistorted region, middle region of distortion, and posterior undistorted area where odontogenic epithelium is growing normally. Note calcio-traumatic bands in incisal dentin. ×21.

Figs. 8 and 9.—Untreated *ia* (Fig. 8) and treated *ia* (Fig. 9) littermates 15 days of age. In Fig. 9 upper incisor shows better organization and much less distortion than in Fig. 8. ×21.

Fig. 10.—Untreated *ia* rat 37 days of age. Note the distortion of the upper incisor and resulting odontome. ×21.

Fig. 11.—Treated *ia* littermate of the animal shown in Fig. 10. Treatment was started at 5 days and a total dosage of 690 I. U. was given. Note absence of odontome, normal periodontal membrane and the eruption of the upper incisor. The slightly distorted incisal tip is the tooth structure formed prior to the treatment. ×10.



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untreated rats. Whereas, in the untreated *ia* rats the first molars showed no signs of root formation, it had started in the above mentioned seven animals and short, irregular roots could be seen. Bone tissue around the cervical and apical parts of teeth was undergoing resorption. A periodontal membrane absent in the untreated *ia* rats, had formed between the alveolar bone and the roots.

In the remaining 3 animals of Group C, which started receiving parathormone at 9 days of age, none of the molars had *completely* erupted. However, the distortion of their odontogenic epithelium and tooth structure was much less marked than in the untreated littermates (Compare Figs. 12 and 15). The root formation of all molars, though distorted, had begun and the teeth had started their eruptive movement toward the oral cavity. In some cases, the second molars had just pierced the oral epithelium (Fig. 15).

It is evident that although all the animals in this group received approximately the same amount of parathyroid hormone, the changes were more marked in the animals in which the administration was started at the age of 4 or 5 days than in those which first received the hormone at 8 or 9 days of age.

Dentin.—The incisal as well as the molar dentin formed during the administration of parathormone showed the characteristic calciotraumatic response[3] (Fig. 7). It consisted of alternating bands of hypo- and hyper-calcification, and their number corresponded to the number of injections given.

TIBIA FINDINGS

1. *Roentgenologic Observations.*—

Group A: The tibia of the untreated *ia* rats showed dense areas of radiopacity in the metaphyseal and epiphyseal regions (Fig. 4).

Group B: Treated *ia* rats of Group B did not show any roentgenologically discernible difference from the untreated *ia* littermate controls.

Group C: In the animals of Group C the radiopaque areas in the proximal and distal metaphyses and epiphyses had either disappeared or were markedly reduced in density (Fig. 5).

2. *Histologic Observations.*—

Group A: The epiphyseal and metaphyseal regions of the untreated tibia showed an absence of resorption of the bony trabeculae and the resulting osteosclerosis (Fig. 16).

Group B: The tibia of the treated rats of Group B, as compared with that of the untreated littermates, did not show any reduction in the number or size of the trabeculae in the metaphyseal or epiphyseal regions. However, there was a very marked change in the cellular elements of the marrow. As compared with the untreated *ia* rats, the hemopoietic cells were markedly reduced in number and in their place masses of spindle cells could be seen (Compare Figs. 16 and 17). These spindle cells filled the areas between the bone trabeculae of the primary spongiosa. On a morphologic basis, the osteoblasts which can easily be differentiated from the surrounding cells in the untreated *ia* rats could not be differentiated from the surrounding spindle cells in the treated rats.

Fig. 12.—Untreated *ia* rat 37 days of age. Note odontomes in the region of all upper molars. None of molars show root formation or eruption. ×15.

Fig. 13.—Treated *ia* rat littermate of that shown in *Fig. 12*. Treatment was begun at 5 days and a total dosage of 690 I. U. was administered. Note normal root formation in all upper molars. Serial sections showed that all molars had erupted. ×15.

Fig. 14.—Treated *ia* rat 45 days of age. Administration of parathormone was begun at 8 days and a total dosage of 615 I. U. was given. Second and third molars have erupted and their root formation (seen here in second molar only) is normal. First molar is less distorted than in Fig. 12, and shows formation of short, though distorted roots. ×15.

Fig. 15.—Treated *ia* rat 42 days of age. Administration of parathormone was begun at 9 days of age and total dosage of 655 I. U. was given. Note partial eruption of first and second molars. Third molar is nearing oral mucosa. Roots of molars are stunted but have formed (compare with Fig. 12). Normal periodontal ligament separates tooth structure from alveolar bone. ×15.

Group C: The tibia of the rats in Group C showed a marked reduction in the amount of osteosclerosis. In the region of the epiphyses the number of bony trabeculae was markedly reduced. The few trabeculae present were much thicker than normal (Compare Figs. 18 and 19).

In the region of the metaphyses, the length and number of the bony trabeculae were considerably reduced, but they were thicker than those seen in the untreated littermate controls. Numerous osteoclasts could be seen around the trabeculae of the primary spongiosa and resorption of bone was in progress.

In addition to the internal resorption of bone tissue, modeling resorption, absent in the untreated rats, could be seen in tibias of Group C. The pattern of resorption, which occurred on the posterior surface of the proximal end and anterior surface of the distal end of the tibia, was normal. The changes in the cellular elements of the marrow were less striking than those seen in Group B. However, the hemopoietic cells in the metaphyseal region of the tibias of Group C were fewer in number than in the litermate controls. The spindle shaped cells described earlier were still present but not in as great numbers as seen in Group B.

DISCUSSION

1. *Effect of Parathyroid Hormone on the ia Anomalies.*—The administration of parathyroid hormone to *ia* rats resulted in a reduction of the incidence and severity of the genetically induced anomalies. The number and size of the pseudo-odontomes in the treated rats were much smaller, and eruption of the molars occurred in many cases. Even the incisors which are most severely affected in *ia* rats erupted in one treated rat which started receiving the injections at 5 days of age, and which received the highest total dosage of parathormone (690 I.U.).

The tibia of the treated rats of Group C approached the normal in its external and internal structure. The modeling resorption could be seen in those areas where it normally occurs, and the number and size of the primary spongiosa in the metaphyses and epiphyses approximated the normal.

2. *Time Factor in Relation to the Effect of Parathyroid Hormone.*—The present study showed that the effect of parathyroid hormone varied with the age at which the administration was started. Thus, in Group B in which all the *ia* rats received about the same amount of parathyroid hormone over the same period of time, the incisors developed more normally in the animals in which the administration was started at 3 days instead of 9 days of life. Similarly in Group C, in which all the *ia* rats received about the same amount of hormone, teeth erupted mostly in those animals in which the administration was started at about 5 instead of 9 days of age. In the *ia* rats the distortion and the ankylosis of the teeth become increasingly severe with age. Therefore, the earlier the administration of parathyroid hormone is started, the more normal is the development of the teeth.

3. *The Effect of Parathormone on Bone Tissue of ia Rats and Grey-Lethal Mice.*—A mutation (grey-lethal) in mice similar to the *ia* rats has been described by Grüneberg.[4] Barnicot[5] studied the effect of parathyroid hormone on the

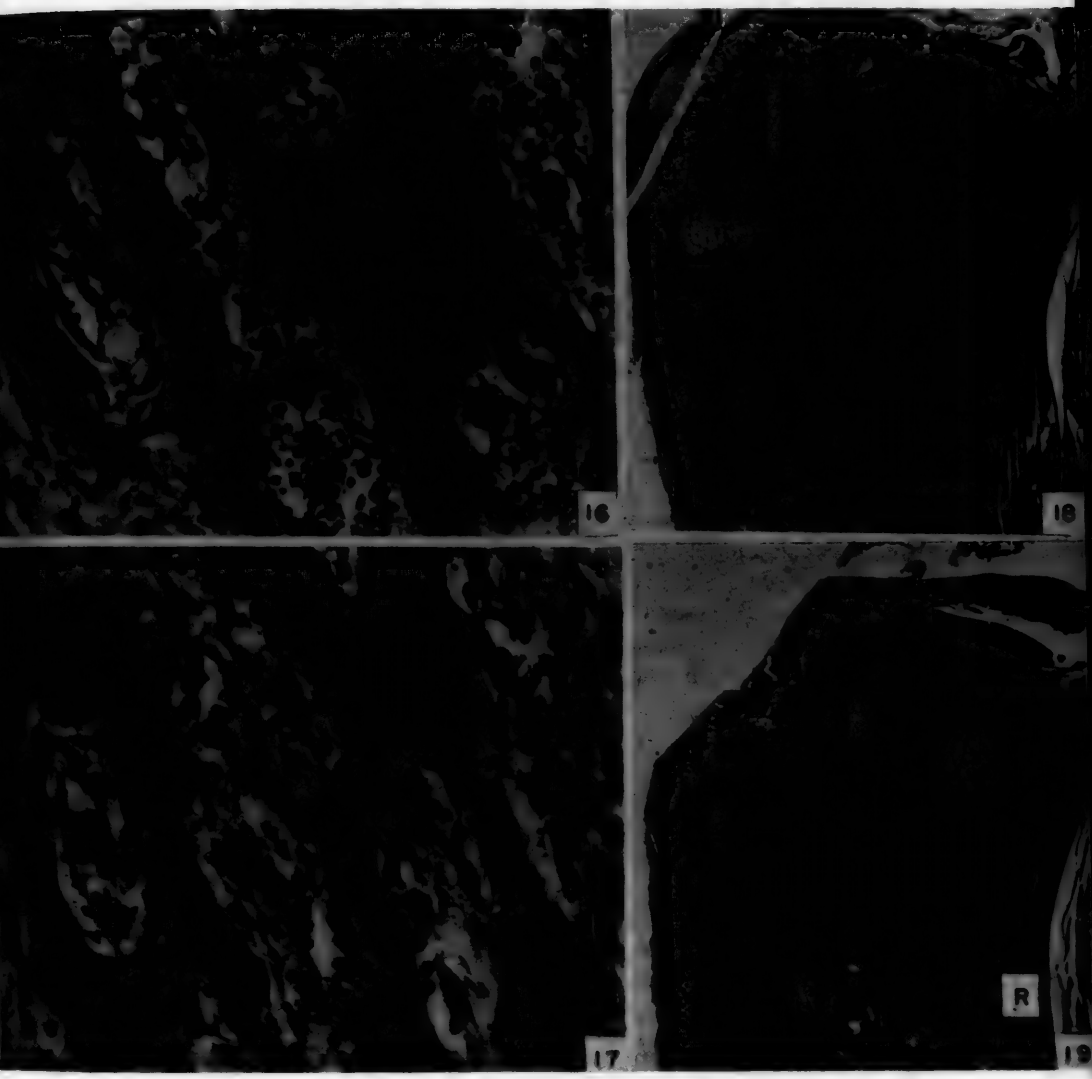

Fig. 16.—Photomicrograph showing cells of bone marrow in metaphyseal region of tibia of untreated *ia* rat 16 days of age. Note osteoblasts lining bone trabeculae and hemopoietic cells in intertrabecular spaces. ×350.

Fig. 17.—Photomicrograph showing cellular elements of marrow in metaphyseal region of tibia of treated *ia* rat, littermate of animal shown in Fig. 16. Note absence of hemopoietic cells and their replacement by numerous spindle shaped cells. ×350.

Figs. 18 and 19.—Photomicrographs of proximal end of tibia in untreated *ia* (Fig. 18) and treated *ia* littermate (Fig. 19). Note reduction in number and increase in thickness of bony trabeculae in Fig. 19. Modeling resorption absent in Fig. 18 can be seen at area R in Fig. 19.

skeleton of the grey-lethal mice but did not observe any improvement in the dental anomalies. He showed that when 2 injections of 70 I.U./100 Gm. of parathormone were administered the resorption of the bone tissue was not striking, but following injections of 7 such doses the resorption was marked. He showed further that low doses (2 to 16 injections of 20-35 I.U. per 100 Gm. of parathormone) led to an "osteoblast phase" and building up of bone in the grey-lethal mice.

Our results agree only in part with those observed by Barnicot. In the present study, it is shown that in the early stages of parathormone treatment (7 injections) there was neither building up nor breakdown of bone tissue in the tibia. On the other hand, resorption of bone was noted in the bone tissue of the dental crypts. In animals which received the parathormone over a longer period of time (33 to 39 injections) resorption of bone occured both in the tibia and around the teeth. It may suggest, therefore, that various regions of the skeleton are not equally affected by small doses of parathormone.

The present investigation also shows that the genetic dental anomalies of the *ia* mutation are curable provided the treatment is started prior to the irreversible ankylosis between the tooth and the surrounding bone.

The discrepancy between the results reported by Barnicot and those described here is apparently the result of two factors: (a) the differences in the chronology of the dental development in the mouse and the rat, and (b) the differences in age at which the administration of the parathormone was started.

Bhaskar, Weinmann, and Schour[6] showed that the incisors, first, second, and third molars of the albino mouse erupt at 10, 15, 18, and 28 days, respectively. Thus, the molars erupt from 4 to 7 days earlier in the mouse than in the rat. Since the most favorable effect of the parathormone is obtained by starting the treatment early in the development of the teeth, the treatment in the mouse would have to be started 4 to 7 days earlier than in the rat to obtain comparable results. Whereas in the present experiment, the administration of parathormone in the rat was started between the second and ninth days of age, Barnicot did not start the treatment until the mice were 6 to 11 days old. Thus, the lack of improvement in the dental anomalies reported by Barnicot was due to a relatively late administration of the hormone.

4. *Calcio-Traumatic Response of the ia Dentin.*—It has been shown that the administration of parathormone[3] results in a characteristic disturbance in the calcification of dentin in the normal rat. This experimental disturbance in the dentin has been called the calcio-traumatic response, and it consists of a sequence of a hypo- and hyper-calcified layer of dentin. The present investigation shows that the *ia* dentin is in this respect similar to the normal dentin.

5. *Spindle Cells.*—The present investigation has shown that in the early stages of the administration of parathormone (Group B) the bone resorption did not occur in the tibia, but the hemopoietic marrow was replaced by masses of spindle cells. The appearance of fusiform or spindle cells in the marrow following the injections of parathormone has been reported previously.[5, 7, 8] Regarding the histogenesis of these cells, Heller, McLean, and Bloom have sug-

gested that they may originate from osteoblast, liberated osteocytes, reticular cells, and osteoclasts. Barnicot[5] and Selye[7] regard these spindle cells as transformed osteoblasts. Since in Group B of the present investigation, there was neither a reduction in the number of osteoclasts, nor any destruction of bone in the tibia, the origin of these cells from the breakdown of osteoclasts or liberated osteocytes, as suggested by Heller, McLean, and Bloom, can be questioned. The possibility that these cells originate from the already differentiated osteoblasts may be ruled out on the basis that their numbers exceed far beyond the number of osteoblasts seen in untreated controls. The most likely origin of these spindle cells, therefore, is the reticular or undifferentiated mesenchymal cells of the bone marrow. Hancox[9] has shown the transformation of histiocytes to osteoclasts. It is, therefore, probable that the first response of the bone marrow to parathyroid hormone is the proliferation of reticular or spindle cells which later differentiate to osteoclasts.

6. *Selective Effect of Parathyroid Hormone.*—Jaffe, Bodansky and Blair[10] showed in the guinea pig that the rapidly growing bones and areas of rapid growth in the same bone are most susceptible to resorption in experimental hyperparathyroidism. That the alveolar bone of the molar area in the rat shows more severe changes following injections of parathyroid hormone than the incisor region, was shown by Weinmann and Schour.[11]

In Group B of the present investigation, it was found that the bone tissue in the upper jaw showed resorption, while no resorption occurred in the tibia. It was shown further that the corrective effect of parathormone on the dental anomalies of the *ia* rat is far more marked in the molar than in the incisor region, and in the upper than in the lower jaw. These findings indicate that different bones may respond differently to the injections of parathormone. That this difference is due only to the differences in the rates of growth of bones seems doubtful.

7. *The Possible Role of the Parathyroid Glands in the ia Mutation.*—On the basis of the present investigation and the findings of Barnicot[5] it seems that an important link in the development of osteosclerosis in the *ia* rats and grey-lethal mice is a retarded development or dysfunction of the parathyroid glands. Recent investigations on a mutation in the rabbit, which resembles the *ia* rats and grey-lethal mice, present further evidence for the involvement of the parathyroid glands.

Pearce and Brown[12, 13] described a recessive, lethal mutation in the rabbit which is characterized by hereditary osteopetrosis of the skeleton, and failure of eruption of teeth. These animals like the grey-lethal mice die within the first five weeks of life and in this respect do not resemble the *ia* rats. Histologic investigation revealed not only the enlargement of the parathyroid glands but the formation of accessory nodules[14] suggesting a state of hyperparathyroidism. On the other hand the serum calcium was found to be low, and serum phosphorus values higher than normal, indicating hypoparathyroidism.[15]

Since in all three mutations (grey-lethal, *ia*, osteopetrosis of rabbits) the osteosclerosis is caused by a lack or retardation of resorption of bone rather

than by an increased bone formation, the assumption of hypofunction of the parathyroid gland seems more justified. The findings of a hyperplasia of the parathyroid gland, as observed by Pearce in rabbits, might reflect a futile attempt to compensate for a qualitative deficiency of parathormone.

Barnicot[16] by reciprocal bone transplantations between the normal and grey-lethal mice has shown that the bone tissue of the grey-lethal mice is normal. Grey-lethal bone tissue transplanted into normal animals was resorbed and normal bones transplanted into grey-lethals became structurally similar to the grey-lethal bone tissue. From these experiments Barnicot concluded that the failure of bone resorption in the grey-lethal mice was due to some changes in the tissue fluids and not to some damage of the bone tissue itself.

Since in the resorption of bone tissue the osteoclasts are supposed to produce an enzyme for the dissolution of the bone matrix, it is quite possible that the *ia* osteoclasts either lack this enzyme or require a higher threshold of parathormone to produce this enzyme.

TABLE I

AGE, NUMBER OF INJECTIONS OF PARATHORMONE, AGE AT START OF THE TREATMENT, TOTAL DOSAGE OF HORMONE RECEIVED AND NUMBER OF ERUPTED TEETH IN 17 *ia* CONTROLS AND 20 TREATED *ia* LITTERMATES

			PARATHYROID ADMINISTRATION			FINDINGS	
GROUP	NUMBER OF ANIMALS	AGE AT SACRIFICE DAYS	NUMBER OF INJECTIONS*	AGE AT START DAYS	TOTAL DOSAGE I.U.	NUMBER OF ERUPTED INCISORS	NUMBER OF ERUPTED MOLARS
A	2	3	–	–	–	–	–
	3	9	–	–	–	–	–
	2	10	–	–	–	–	–
	4	15	–	–	–	–	–
	3	37	–	–	–	–	–
	3	42	–	–	–	–	–
B	1	8	7	2	125	–	–
	3	10	7	3	140	–	–
	4	15	7	9	140	–	–
C	1	37	33	5	690	3	12
	3	42	34	9	655	–	–
	6	43	39	4	655	–	4-7
	2	45	38	8	615	–	3-4

*Treatment consisted of daily injections of 10, 15, 20 and 25 international units of parathormone given in progressively increasing doses.

SUMMARY AND CONCLUSIONS

1. This roentgenologic and histologic study deals with the effect of parathormone on the dentition and the tibia of the *ia* rats which are characterized by hereditary hypo-osteoclasis and failure of eruption of teeth. Animals were divided into three groups—Group A consisted of 17 *ia* rats which were used as controls; Group B of 8 *ia* rats which were given 7 injections of parathormone (total dosage 140 I. U.); and Group C of 12 *ia* rats which received 33 to 39 injections of parathormone (total dosage 615 to 690 I. U.).

2. The administration of parathormone resulted in the reduction of the incidence and severity of the dental anomalies, pseudo-odontomes, and in most of the animals of Group C, in the eruption of teeth. The favorable effect of

parathormone on the *ia* dentition was correlated with the amount given and the time at which the administration was started. The animals in which administration was started early and continued longest showed normal development and eruption of the largest number of teeth.

3. Animals receiving 7 injections of parathormone did not show reduction of osteosclerosis in the tibia. However, there was a marked change in the cellular elements of the marrow. Masses of spindle cells were seen in place of hemopoietic cells. The *ia* rats which received 33 to 39 injections of parathormone showed the beginning of normal modeling resorption and marked reduction of the osteosclerosis in the metaphyses and epiphyses of the tibia. The number of spindle cells had diminished.

Fig. 20.—Camera lucida drawings of the upper incisors and proximal ends of the tibia of normal, *ia*, and treated *ia* rats. Note the corrective effect of parathormone on the *ia* anomalies.

4. The investigation demonstrates the corrective effect of parathormone on the anomalies of the *ia* mutation (Fig. 20) and thus also sheds some light on the mechanism of the mutation.

The authors wish to express their thanks to Professor Harry Sicher of the Loyola University School of Dentistry for his helpful suggestions. To Mr. William Winn, biologic photographer, we are grateful for preparing the illustrations.

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THE EFFECT OF ACTH AND CORTISONE ON THE SODIUM AND POTASSIUM LEVELS OF HUMAN SALIVA

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ALL CELLS and tissue fluids contain sodium and potassium. Despite the widespread distribution of these elements and the changes in concentration which may occur in a variety of metabolic disorders, the physiologic role of sodium and potassium is not yet completely understood. In an attempt to elucidate their function in human metabolism, blood, tissues, and excretions are being analyzed for these elements before, during, and after the administration of various therapeutic agents at the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama. Like others, we have observed that the adrenal cortex exerts a profound influence on the metabolism of sodium and potassium. Abnormalities of adrenal cortical function result in readily detectable changes in the blood levels of these elements. Acute adrenal cortical hypofunction (crisis of Addison's disease) produces a decrease in serum sodium concentration and an increase in serum potassium concentration. Adrenal cortical hyperfunction (Cushing's syndrome) is usually accompanied by a decrease in the serum potassium level and inconstantly by an increase in the serum sodium content. The administration of ACTH or cortisone to patients with adrenal cortical insufficiency reverses somewhat the changes in the blood levels of sodium and potassium.

Sodium and potassium are present in human saliva in quantities which reflect those contained in the circulating blood. This relationship is not absolute. It may be limited by differences in the permeability of the secretory cells of the salivary glands to the various ions present in blood, and by differences in the response of the salivary glands to different stimuli and to different intensities of the same stimulant. The sodium and potassium concentrations in resting and stimulated saliva are thus characterized by a high degree of individual variation. Indicative of this variability is the wide range of mean sodium and potassium levels found in resting human saliva by Clark and Shell,[1] Beeks,[2] and Mathis.[3] These consisted of 51, 70, and 30 mg. per cent, respectively, for sodium and 63, 56, and 71 mg. per cent, respectively, for potassium. In stimulated saliva, Brown and Klotz[4] observed values which ranged from 16.5 to 131 mg. per cent for sodium and from 57 to 115 mg. per cent for potassium.

That the saliva concentrations of sodium and potassium may be influenced by the adrenal cortex is suggested by the reports of Grad,[5] Frawley and Forsham,[6] and Frawley and Thorn[7] who observed a consistent reduction in

This study was supported by grants from the Clayton Foundation for Research.
Received for publication, Dec. 6, 1951.

the sodium/potassium ratio in unstimulated mixed saliva following the administration of ACTH or desoxycorticosterone acetate to patients with adrenal cortical hypofunction. Because of the consistency of this reduction, it has been recommended as a basis for a cortical assay technique.[8] It thus appears that the composition of human saliva may be under hormonal as well as neural control. Since the total percentage of inorganic salts in saliva is directly related to the rate of secretion,[9] it seemed of interest to determine whether the saliva sodium and potassium-regulating action of ACTH and synthetic cortisone acetate in cases of adrenal cortical hypofunction is independent of the rate of flow.

MATERIALS AND METHODS

Five patients with a slight to severe degree of acute rheumatoid arthritis were selected for this study. This group consisted of 2 males and 3 females, ranging in age from 31 to 65 years. Three of these patients had been treated previously with ACTH or cortisone and on each occasion the administration of these agents had resulted in a prompt though temporary remission of the arthritic symptoms.

Samples of both resting and paraffin-stimulated mixed saliva were collected from each patient at 8:00 A.M. and at 1:00 P.M. before, during, and after treatment with ACTH or cortisone. The pretreatment period consisted of 5 days during which base line determinations were made. The period of therapy comprised 7 to 11 days and the posttreatment period, 6 to 12 days. Patients M. A., R. H., and O. G. received a total of 1,300, 1,625, and 1,650 mg. of synthetic cortisone acetate respectively. Patients S. B. and J. D. were given a total of 750 and 1,000 mg. of ACTH, respectively. The dosage was adjusted from day to day in accordance with the needs of each patient (Fig. 1). The hormones were injected intramuscularly in divided doses (b.i.d.), the first shortly after the collection of the morning samples of saliva and the second 6 hours later. In each instance the administration of these agents was terminated when a clinical remission of the arthritic symptoms was well under way. The clinical response to therapy in each patient was similar to that described by Spies and Stone.[10]

The morning samples of saliva were obtained before breakfast and before brushing the teeth. Each patient was instructed to refrain from taking any other medication during the period of study. No restrictions were placed on the salt intake of these patients. Sodium- and potassium-free blocks of paraffin served as secretory stimulants. Each morning and afternoon sample of resting and stimulated saliva consisted of approximately 5 c.c. which were collected in specially cleaned Pyrex tubes. The sodium and potassium content was determined by flame photometry. Each sample of saliva was centrifuged and the supernatant liquid diluted until it contained between 1 and 10 ppm of sodium or potassium. After adding the internal standard element (500 ppm Li), sodium and potassium analyses were performed by means of the Perkin-Elmer Model 52-A flame photometer. In making these determinations, the general procedure of Bills, McDonald, Niedermeier, and Schwartz[11] was fol-

TABLE I

SODIUM AND POTASSIUM LEVELS OF 8:00 A.M. SALIVA SAMPLES

PATIENT	MEAN NA (R)*	RANGE	MEAN NA (S)†	RANGE	MEAN K (R)*	RANGE	MEAN K (S)†	RANGE
Pretreatment Values (Mg. Per Cent)								
1. M. A.	119.2	60-195	171.6	105-205	66.4	53-78	65.8	60-78
2. S. B.	25.6	18-34	77.0	54-91	111.6	85-125	106.8	100-118
3. J. D.	30.3	15-61	93.2	66-111	75.2	63-85	70.8	58-81
4. O. G.	27.7	23-31	31.3	29-35	79.3	69-98	72.0	65-76
5. R. H.	29.8	21-38	52.2	45-60	87.7	74-100	83.0	74-89
Values During Therapy (Mg. Per Cent)								
1. M. A.	68.6	51-95	141.4	30-190	80.1	70-88	69.0	61-73
2. S. B.	42.0	31-56	66.6	36-124	118.9	103-133	90.5	61-109
3. J. D.	74.6	38-101	91.2	69-103	96.1	56-133	91.2	89-160
4. O. G.	21.5	13-29	29.6	22-39	72.3	61-91	73.1	65-79
5. R. H.	30.6	21-46	37.4	26-55	83.9	73-95	78.5	63-93
Posttreatment Values (Mg. Per Cent)								
1. M. A.	64.2	53-88	142.4	40-180	74.4	60-83	66.1	55-70
2. S. B.	46.6	38-61	75.0	66-86	126.3	116-145	96.5	86-105
3. J. D.	68.7	29-92	96.0	66-125	83.9	53-116	67.1	56-79
4. O. G.	16.3	11-25	18.7	18-19	78.3	71-84	77.0	75-78
5. R. H.	31.5	28-38	44.3	29-53	72.6	66-81	70.7	65-74

*(R)—Resting saliva.
†(S)—Stimulated saliva.

lowed using the internal standard technique of Berry, Chappel, and Barnes.[12] The observed sodium and potassium contents of each sample were calculated as milligrams per cent and milliequivalents per liter of saliva. Sodium/potassium ratios were established for each sample analyzed.

TABLE II

SODIUM AND POTASSIUM LEVELS OF 1:00 P.M. SALIVA SAMPLES

PATIENT	MEAN NA (R)*	RANGE	MEAN NA (S)†	RANGE	MEAN K (R)*	RANGE	MEAN K (S)†	RANGE
Pretreatment Values (Mg. Per Cent)								
1. M. A.	82.0	63-108	186.6	175-195	52.0	48-60	65.0	65-65
2. S. B.	20.3	18-23	66.3	59-74	131.3	108-153	124.3	108-133
3. J. D.	27.7	23-31	77.7	55-90	79.0	69-89	79.0	74-84
4. O. G.	22.0	18-26	31.0	31-31	72.0	71-73	85.5	80-91
5. R. H.	22.0	18-30	51.7	46-59	77.3	70-81	86.3	69-99
Values During Therapy (Mg. Per Cent)								
1. M. A.	45.7	22-63	146.1	118-173	60.7	48-70	68.4	60-75
2. S. B.	16.3	12.5-19	53.2	46-66	121.2	106-130	117.7	111-123
3. J. D.	13.7	9-20	57.1	39-74	103.5	90-124	86.5	81-99
4. O. G.	13.1	5-23	18.7	14-31	89.0	74-112	85.3	76-93
5. R. H.	17.6	10-35	34.6	19-49	80.2	63-94	78.2	71-81
Posttreatment Values (Mg. Per Cent)								
1. M. A.	38.2	23-55	146.3	125-168	56.3	35-70	68.3	63-75
2. S. B.	21.9	18-28	56.2	36-70	116.5	71-158	114.2	98-132
3. J. D.	35.0	19-56	106.4	66-125	78.0	71-89	73.6	63-84
4. O. G.	17.7	14-25	17.7	15-19	93.7	86-100	85.5	75-96
5. R. H.	28.6	9-41	42.0	17-58	77.8	70-90	83.6	74-98

*(R)—Resting saliva.
†(S)—Stimulated saliva.

TABLE III

THE SODIUM AND POTASSIUM CONTENT OF RESTING AND STIMULATED SALIVA BEFORE, DURING, AND AFTER TREATMENT WITH ACTH OR CORTISONE

PERIOD	TIME	SAMPLES	RESTING SALIVA				STIMULATED SALIVA			
			MEAN NA (MG. %)	RANGE NA (MG. %)	MEAN K (MG. %)	RANGE K (MG. %)	MEAN NA (MG. %)	RANGE NA (MG. %)	MEAN K (MG. %)	RANGE K (MG. %)
Pretreatment	8 A.M.	24	47.3	15-195	78.5	53-125	87.3	29-205	80.0	58-118
	1 P.M.	14	38.1	18-108	83.8	48-153	86.3	31-195	88.2	65-133
Treatment	8 A.M.	41	44.1	13-101	88.2	56-133	65.9	22-190	81.9	61-160
	1 P.M.	45	20.6	5-63	90.3	48-130	57.2	14-173	87.3	60-123
Posttreatment	8 A.M.	37	51.3	11-88	92.8	53-145	91.0	18-180	76.2	55-105
	1 P.M.	31	29.4	9-56	87.6	35-158	90.6	15-168	85.8	63-132

OBSERVATIONS

The Relative Concentration of Sodium and Potassium in Human Whole Saliva.—The mean sodium and potassium levels of the morning and afternoon samples of resting and stimulated saliva of each of the 5 patients before, during and after treatment with ACTH or synthetic cortisone are shown in Tables I and II. The mean sodium levels of unstimulated morning saliva exceeded those of unstimulated afternoon saliva in each patient during each phase of the study. A similar but less constant relationship was observed in the saliva obtained by paraffin stimulation. The mean sodium values of stimulated saliva were considerably higher than those of unstimulated saliva both in the morning and afternoon. In contrast, the mean potassium levels of resting saliva in each patient exceeded those of stimulated saliva in the 8:00 A.M. samples. The mean potassium levels of unstimulated saliva were higher than the mean sodium levels in each patient in both the morning and afternoon samples. A similar but inconstant trend was noted for stimulated saliva.

The sodium and potassium levels of each saliva sample obtained from each patient were pooled and analyzed collectively (Table III). It will be noted that the sodium and potassium content of the samples extended over an exceedingly wide range before, during and after treatment with ACTH or cortisone. The mean potassium content of the entire group of samples of unstimulated saliva greatly exceeded that of sodium both in the morning and afternoon during each of the three phases of study. This relationship was not observed in the samples of stimulated saliva except during the period of treatment. The mean sodium levels of resting and stimulated saliva decreased during treatment with ACTH or cortisone and increased when therapy was discontinued. These changes were of a greater magnitude in the afternoon samples than in the morning samples. In contrast, there was a slight increase in the mean potassium concentration in the morning and afternoon samples of unstimulated saliva without a corresponding change being noted in the samples of stimulated saliva.

The Effect of Cortisone or ACTH on the Relative Concentration of Sodium and Potassium in Human Whole Saliva.—The administration of ACTH or cortisone resulted, in each case, in a general decrease in the mean sodium values of stimulated morning saliva and of both unstimulated and stimulated afternoon saliva as contrasted with the values prevailing during the pretreatment period. In most instances, the mean sodium values in each patient increased toward, or even exceeded, the pretreatment levels following the discontinuation of therapy. The changes in the potassium values during the period of therapy were not as consistent nor as pronounced as those for sodium. These changes are shown in Figs. 1 and 2.

In each of the 192 samples of unstimulated saliva and 192 samples of stimulated saliva, the potassium concentration greatly exceeded the amount normally present in blood serum or plasma (16 to 22 mg. per cent). In contrast, the sodium concentration was from 3 to 15 times lower in saliva than in blood plasma or serum (300 to 330 mg. per cent).[13] The potassium content of un-

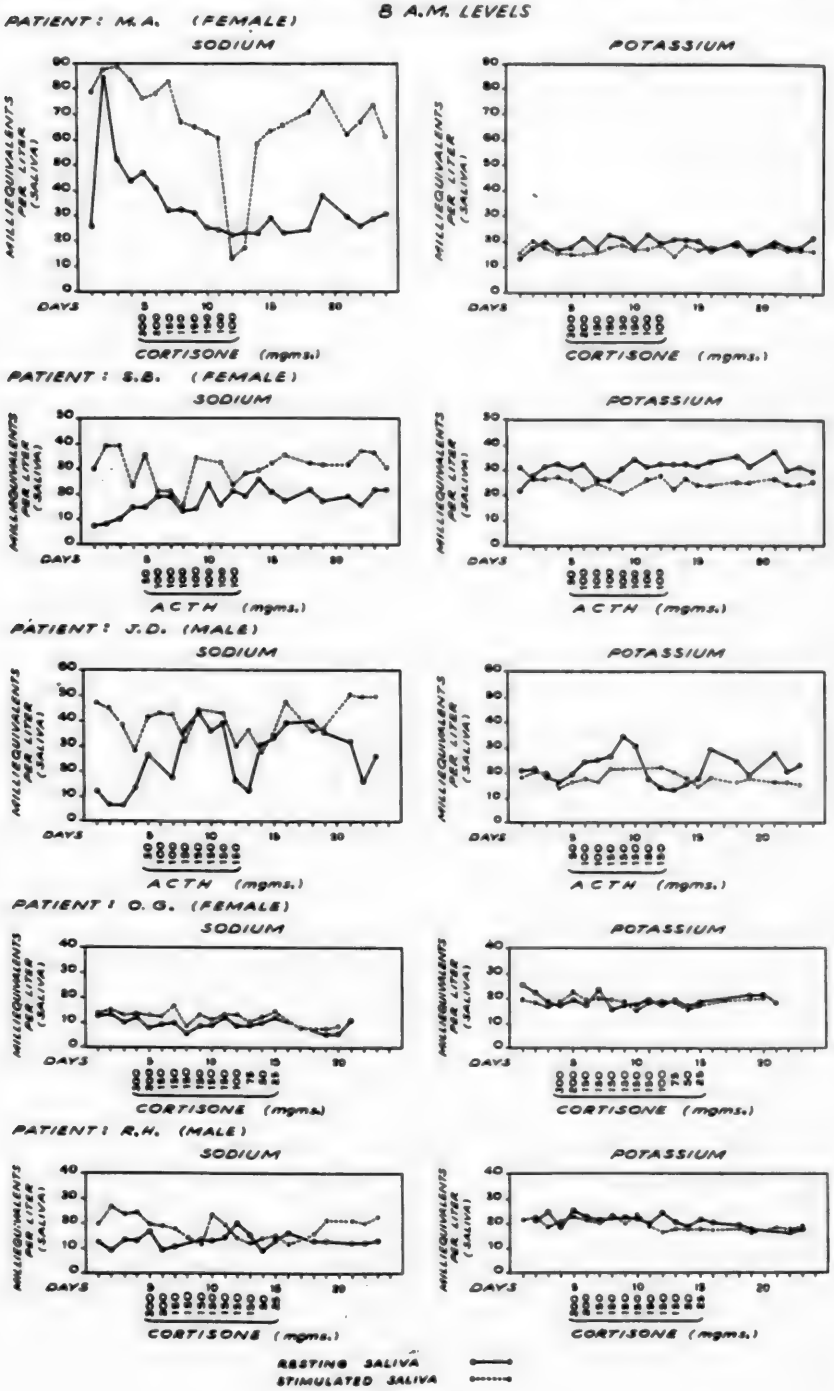
THE EFFECT OF ACTH AND CORTISONE
ON SODIUM AND POTASSIUM LEVELS OF WHOLE SALIVA
8 A.M. LEVELS
PATIENT : M.A. (FEMALE)
SODIUM
POTASSIUM
MILLIEQUIVALENTS PER LITER (SALIVA)
DAYS
CORTISONE (mgms.)
PATIENT : S.B. (FEMALE)
ACTH (mgms.)
PATIENT : J.D. (MALE)
PATIENT : O.G. (FEMALE)
PATIENT : R.H. (MALE)
RESTING SALIVA
STIMULATED SALIVA

Fig. 1.

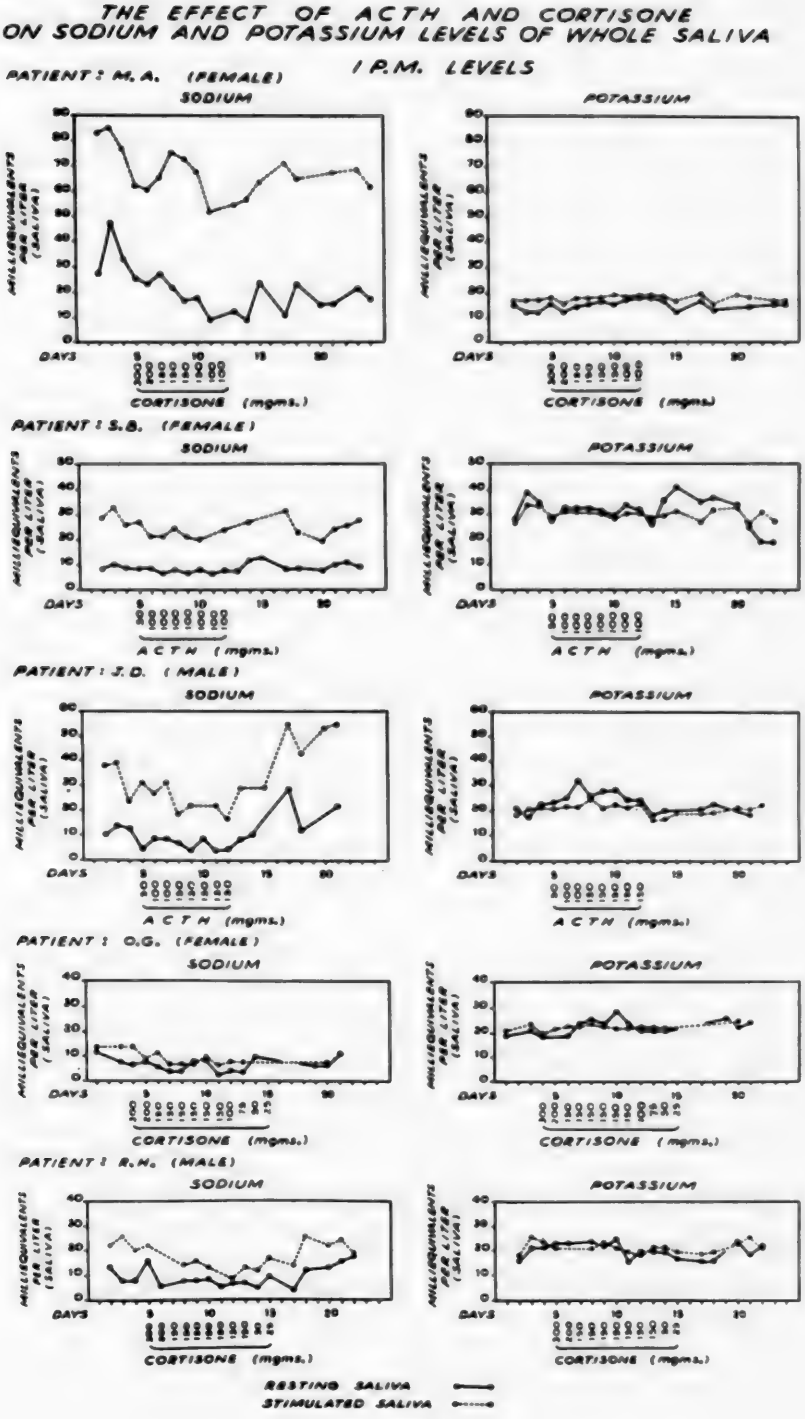
THE EFFECT OF ACTH AND CORTISONE
ON SODIUM AND POTASSIUM LEVELS OF WHOLE SALIVA
1 P.M. LEVELS
PATIENT : M. A. (FEMALE)
SODIUM
POTASSIUM
MILLIEQUIVALENTS PER LITER (SALIVA)
DAYS
CORTISONE (mgms.)
PATIENT : S.B. (FEMALE)
SODIUM
POTASSIUM
ACTH (mgms.)
PATIENT : J.D. (MALE)
SODIUM
POTASSIUM
ACTH (mgms.)
PATIENT : O.G. (FEMALE)
SODIUM
POTASSIUM
CORTISONE (mgms.)
PATIENT : R.H. (MALE)
SODIUM
POTASSIUM
CORTISONE (mgms.)
RESTING SALIVA
STIMULATED SALIVA

Fig. 2.

stimulated and stimulated morning and afternoon saliva exceeded that of sodium in three of the five patients before, during and after treatment with ACTH or synthetic cortisone acetate. In one patient (J. D.) this relationship prevailed in all samples of unstimulated saliva but was reversed in the morning and afternoon samples of stimulated saliva obtained during the pre- and post-treatment periods. The sodium content of unstimulated and stimulated morning and afternoon saliva in the fifth patient (M. A.) exceeded the potassium content with the exception of those samples of unstimulated saliva collected while the patient was receiving injections of cortisone.

The Effect of ACTH or Cortisone on the Sodium/Potassium Ratio of Human Whole Saliva.—The effects of ACTH and cortisone on the sodium/potassium ratios of the morning and afternoon samples of resting and stimulated saliva are shown in Fig. 3. A decreased sodium/potassium ratio was observed in the 1:00 P.M. samples of resting and stimulated saliva in each of the 5 patients who received either ACTH or synthetic cortisone acetate by intramuscular injection. Cessation of therapy was followed by a change in the sodium/potassium ratio which approached pretreatment levels. In one patient (M. A.) the decreased sodium/potassium ratio in the stimulated saliva was very slight. The decrease of the sodium/potassium ratio in unstimulated saliva greatly exceeded that in stimulated saliva in each patient. No consistent changes were noted in the sodium/potassium ratios of the 8:00 A.M. samples of resting or stimulated saliva of the patients receiving cortisone. An increased sodium/potassium ratio during the period of therapy was observed in the morning samples of unstimulated saliva of the two patients, J. D. and S. B., who received ACTH. These ratios failed to return to pretreatment levels throughout the posttreatment period.

With but 9 exceptions, the sodium content of the 192 samples of stimulated morning and afternoon saliva greatly exceeded that of the samples of unstimulated saliva collected during the same time of day throughout each period of study. The potassium content either remained of the same order of magnitude or underwent a slight reduction during stimulation. The net result of stimulation of the salivary glands was an increase in the rate of flow characterized by an increase in the combined percentage of sodium and potassium ions present in each sample. This increase was due in almost all instances to the increased content of sodium. The decrease in the sodium/potassium ratios of the afternoon samples of resting and stimulated saliva during the period of administration of ACTH or cortisone resulted primarily from a decrease of the sodium content of the samples below the pretreatment levels without a corresponding change in the potassium values. This effect was not observed in the samples of resting or stimulated morning saliva obtained during the treatment period.

SUMMARY AND CONCLUSIONS

1. The repeated intramuscular injection of pituitary ACTH or synthetic cortisone is followed by definite changes in the concentration of sodium in the human saliva. The sodium level of resting and stimulated saliva declined within 4 hours after the beginning of injections in each of the 5 subjects.

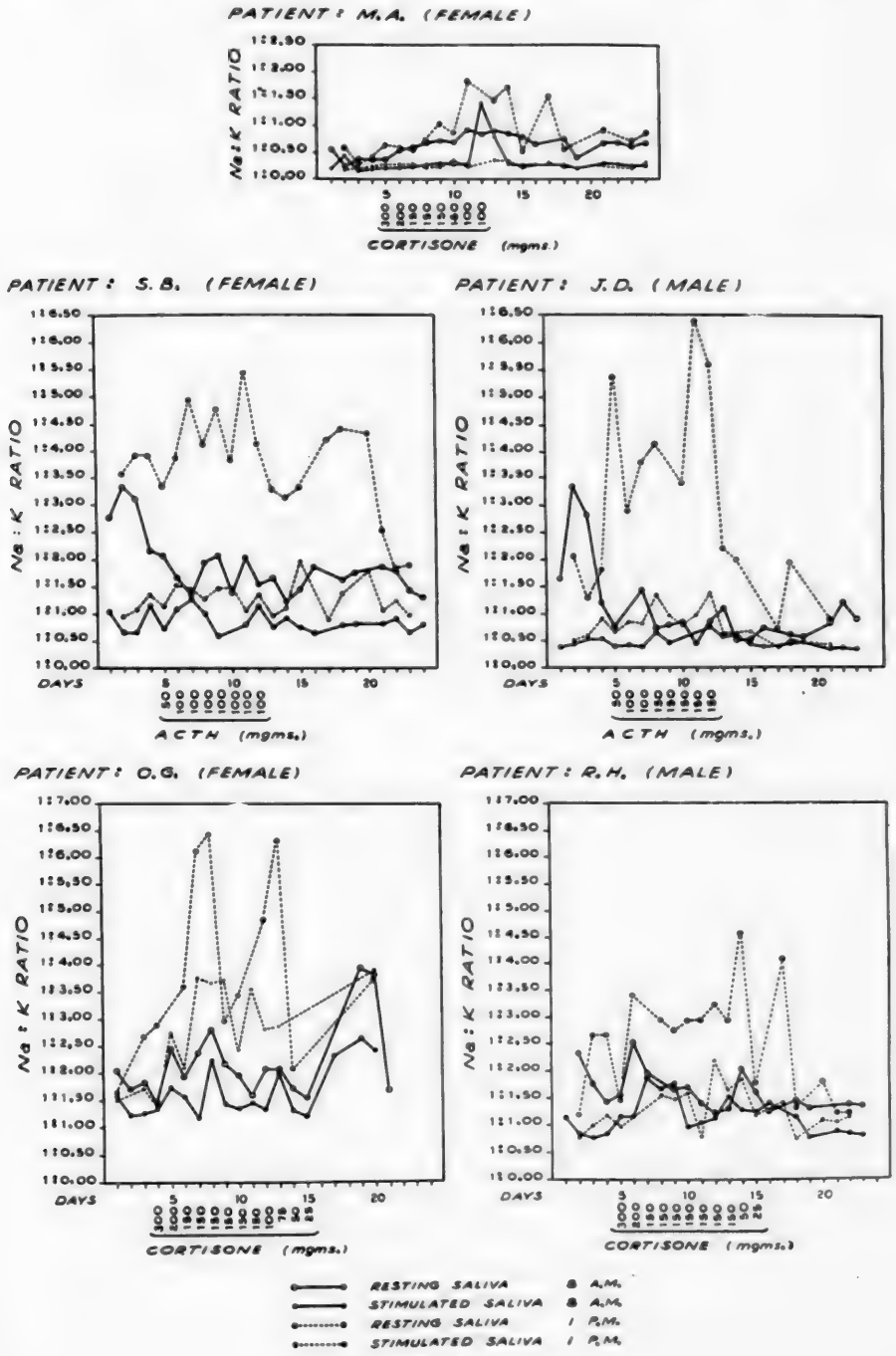
THE EFFECT OF ACTH AND CORTISONE
ON SODIUM / POTASSIUM RATIO OF WHOLE SALIVA
PATIENT : M.A. (FEMALE)
Na : K RATIO
CORTISONE (mgms.)
PATIENT : S.B. (FEMALE)
PATIENT : J.D. (MALE)
Na : K RATIO
DAYS
ACTH (mgms.)
PATIENT : O.G. (FEMALE)
PATIENT : R.H. (MALE)
CORTISONE (mgms.)
RESTING SALIVA 8 A.M.
STIMULATED SALIVA 8 A.M.
RESTING SALIVA 1 P.M.
STIMULATED SALIVA 1 P.M.

Fig. 3.

2. The potassium concentration of the saliva was not materially altered following the injection of either of these hormones in any of the subjects with the result that there was a decrease in the sodium/potassium ratio. The decrease of the sodium/potassium ratio was greater in the samples of resting saliva as contrasted with the samples of stimulated saliva. This difference appears related to the difference in the rate of flow.

3. The potassium content greatly exceeded the sodium content in the samples of saliva before, during and after treatment with either ACTH or synthetic cortisone acetate (Table III). In every patient the potassium content of the saliva exceeded the normal blood serum or plasma value for this element. In contrast, the blood sodium content in each patient was approximately 3 to 15 times as great as the sodium concentration of the saliva.

4. The present studies show that saliva is not an ultrafiltrate of the blood. The secretory cells of human salivary glands possess the faculty of selecting and concentrating certain blood constituents in their elaboration of saliva, a process which is under hormonal as well as neural control.

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AMMONIA PRODUCTION AND UREASE ACTIVITY IN SALIVA

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AMMONIA concentration in saliva has been suggested as one of the controlling factors in natural immunity to dental caries. Although the ammonia concentration has been thoroughly investigated,[1-4] in no case has it been reported as high as the 50 mg. per cent claimed by Kesel[5] to be necessary for the inhibition of the growth of lactobacillus acidophilus. Ludwick and Fosdick[6] investigated the ammonia content of dental brushings to determine whether the ammonia concentration on the tooth itself was related to dental caries. No difference was found in the ammonia concentration on the tooth surfaces of caries-active or caries-immune persons. Kesel[5] measured the ammonia produced by broth cultures of caries-immune salivas over an 8 day period and found that the caries-immune broths had an inhibitory effect on the growth of lactobacillus acidophilus which he attributed to the high ammonia content.

In a previous report[7] we found the ammonia producing ability of saliva reached a maximum in 24 to 48 hours and in no case did the amount produced equal 50 mg. per cent. As the salivas of a limited number of individuals formed the basis of this observation, it was decided to extend the investigation to a larger group and to include a study of the relation of the amount of salivary urease to the lactobacillus count and the effect of glucose on the activity of salivary urease.

EXPERIMENTAL

Salivas were collected from 68 students at the Faculty of Dentistry, University of Toronto. The lactobacilli were counted* and the salivas incubated for 24 hours at 37° C., after which the ammonia N content was determined by the Folin-Bell permutit method.[8] The color was measured in a Lumetron colorimeter.

TABLE I

MG. PER CENT NH_3-N PRODUCED IN SALIVA IN 24 HOURS

	LACTOBACILLUS COUNTS		
	UNDER 100	100-50,000	OVER 50,000
No. of Samples	20	25	21
Range of Values (mg.% NH_3-N)	16.7-42.5	11.1-63.0	11.5-56.0
Mean	29.2	27.6	32.6
Standard Deviation	10.1	11.0	13.7

The results of Table I show no significant relationship between ammonia produced and lactobacillus count. All values fall within the same broad range and exhibit a high standard deviation.

Received for publication, Nov. 16, 1951.

*Lactobacillus counts were made by Miss Berry, Faculty of Dentistry, University of Toronto.

Hine and O'Donnell[9] found that in most cases salivary samples with low lactobacillus counts contained more active urease-producing bacteria than those with high counts. We were concerned with the relationship between the urease activity of the salivas and the lactobacillus counts. This was measured by adding a solution of urea to the saliva to give a concentration of 1.5 per cent and then incubating the mixture for 24 hours at 37° C. The ammonia liberated in 24 hours was measured by means of the previous method.[8] The results are shown in Table II.

TABLE II

MG. PER CENT NH_3-N PRODUCED IN 24 HOURS FROM UREA-SALIVA MIXTURES

	LACTOBACILLUS COUNTS		
	UNDER 100	100-50,000	OVER 50,000
No. of Samples	19	25	20
Range of Values (mg.% NH_3-N)	72-382	34-380	56-430
Mean	175	163	200
Standard Deviation	72	91	97

In considering the combined data of Tables I and II there was some indication of a relationship between the amount of ammonia produced in saliva in 24 hours and the amount of ammonia produced when urea was added to the saliva. When these amounts were plotted the points were scattered around a straight line in such a way as to suggest correlation. However, calculation of the correlation coefficient proved that this relationship was not a significant one.

In our previous paper[7] the stimulatory effect of glucose on the action of salivary urease was noted. Extending this initial study to a larger number of subjects it was found that in all cases a concentration of 2.5 per cent glucose increased the production of ammonia in saliva-urea mixtures. The results are shown in Table III.

TABLE III

MG. PER CENT NH_3-N PRODUCED IN 24 HOURS FROM UREA-SALIVA MIXTURES PLUS 2.5 PER CENT GLUCOSE

	LACTOBACILLUS COUNTS		
	UNDER 100	100-50,000	OVER 50,000
No. of Samples	17	25	20
Range of Values (mg.% NH_3-N)	62-820	73-712	152-970
Mean	400	346	533

From the results of Table III it can be seen that the amounts of ammonia formed do not appear to be related to the lactobacillus counts.

SUMMARY

1. The activity of the ammonia-producing mechanism appears to be unrelated to caries activity as measured by lactobacillus counts.

2. There is no direct relationship between the urease content of saliva and the lactobacillus count.

3. The relation between urease activity and ammonia production is not a significant one.

4. In all cases the presence of 2.5 per cent glucose was found to stimulate the activity of the urease present in saliva.

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ALKALINE PHOSPHATASE IN THE JAW BONES AND TEETH OF THE ALBINO RAT AS RELATED TO ABNORMAL STATES OF BODY GROWTH, MINERAL METABOLISM, AND DEVELOPMENT

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THE spatial arrangement of the enzyme alkaline phosphatase in the tissues adjacent to calcified structures has been the subject of many investigations since the advent of the Gomori histochemical technique.[1] Kabat and Furth[2] first demonstrated the presence of alkaline phosphatase in the tissues surrounding teeth and bones. Subsequently, detailed studies have been published describing the distribution and localization of alkaline phosphatase in the developing teeth by Gomori,[3] Engel and Furuta,[4] Horowitz,[5] Bevelander and Johnson,[6] and Morse and Greep[7] and in the bones by Lorch,[8] Zorzoli,[9] and Greep, Fischer, and Morse.[10]

These investigations of the histochemical distribution of alkaline phosphatase suggest that the enzyme plays a role in the formation of the hard elements of the teeth and bones. The phosphate radicals which are split from the organophosphates by phosphomonoesterases and become incorporated, in part at least, in the calcium compounds peculiar to hard structures. It would seem, therefore, that the enzyme is indispensable to normal calcification.[11]

It was felt that elucidation of the metabolic role of phosphatase might be aided by determining its histochemical localization under conditions of altered body growth, altered mineral metabolism, and abnormal development. Consequently, the phosphatase complement of the teeth and jaw bones of the albino rat was studied in hypophysectomized and rachitic animals and in a mutant strain of rats (''ia'')[15] with a transitory defect in bone resorption.

MATERIALS AND METHODS

Thirty-five female rats were hypophysectomized at 25 to 28 days of age. Groups of 4 or 5 of these animals were sacrificed at intervals of 14, 21, and 32 days following the operation. Other groups of hypophysectomized animals were injected with 1 mg. of Armour's growth hormone per day for 1, 2, or 3 days following a postoperative period of 14 days. Normal controls of comparable age were sacrificed at the same time.

A group of 35 male and female animals was placed on a synthetic rachitogenic diet (Table 1) at 21 to 23 days of age and sacrificed without regard to sex in groups of 6 or 7 after 14, 21, 26, 36, and 125 days.

Rats of the ''ia'' (incisor-absent) strain were sacrificed at birth and at 2, 5, 10, 15, 20, and 30 days postnatally.

This work was done under a grant from the American Cancer Society, Inc., on the recommendation of the Committee on Growth of the National Research Council.

Received for publication, Nov. 30, 1951.

All the rats were fed purified diets (Table I). The control diet, adequate in all known constituents, consisted of sucrose, vitaminized lactalbumin, vitaminized corn oil and calcium, and phosphorus-deficient salts to which calcium carbonate and dibasic sodium phosphate were added to obtain 0.52 per cent calcium and 0.37 per cent phosphorus. The constituents of the rachitogenic diet appear in the second column of Table I. Vitamin D was eliminated from the corn oil and the calcium content was increased to 1.04 per cent while the amount of phosphorus was decreased to 0.09 per cent.

TABLE I

CONSTITUENTS OF THE DIETS

	NORMAL DIET (GM.)	RACHITOGENIC DIET (GM.)
Sucrose	335	335
Lactalbumin plus crystalline vitamin B complex*	120	120
Corn oil plus fat-soluble vitamins*:		
with vitamin D	25	—
without vitamin D	—	25
Salt mixture deficient in calcium and phosphorus*	11	11
Calcium carbonate	6.5	13
Dibasic sodium phosphate	8.5	2

*Shaw.

The heads of all the animals were split along the midsaggital plane immediately after removal. One-half head was placed in 10 per cent formaldehyde in preparation for routine histological examination; the other half head was fixed in chilled 80 per cent ethyl alcohol for 24 hours, decalcified, embedded and stained according to our standardized technique for the demonstration of alkaline phosphatase in the bones and teeth of older animals.[10] Sections were made of the upper incisor in a longitudinal plane, the upper molars in mesiodistal plane, the lower incisor in cross section at the level of the third molar, and the mandibular condyle in a vertical plane.

RESULTS

Normal Control.—The distribution and localization of alkaline phosphatase in the control animals were identical with those found in the normal animals of previous studies.[6, 7, 9, 10] In the bone of the alveolar process, phosphatase was found in the endosteal and periosteal tissue, Sharpey's fibers, the more peripheral osteocytes, and the marrow cells. A faint reaction appeared in the newly apposed matrix. In the incisors, the reduced enamel organ and the pulp cells immediately subjacent to the osteoblasts, especially on the labial aspect of the tooth, contained the greatest concentration of enzyme. Enzyme was also present in the amelobasts just previous to and during mineralization of the enamel matrix, the pulp cells and the periodontal membrane (Fig. 1).

The localization of phosphatase in the molars was essentially the same as that in the incisor, although the distribution varied slightly due to the morpho-

logical differences between the two types of teeth (Fig. 10). The pulp of the molar teeth contained phosphatase throughout with the greatest concentration occurring in the coronal horns. The odontoblastic processes in the same area were characteristically darker and more pronounced than elsewhere. The mucosa was devoid of enzyme but the cells of the epithelium of the interdental papillae and the free margin of the gingiva contained varying amounts of phosphatase. All the nuclei were faintly positive with scattered cells exhibiting coarse, granular, reactive areas in the cytoplasm. As elsewhere, the reactions of the components of the alveolar and supporting bone, the periodontal membrane and the vascular elements were typical.

The mandibular condyle of the control animals at the start of the experiment, i.e., 25 days, was capped by a cartilage consisting of three distinct zones. The embryonic zone consisted of a narrow band of flattened cells on the articulating surface. A faint phosphatase reaction appeared only in the nuclei of these cells. The second zone made up of enlarged, rounded cells exhibited enzyme in both nuclei and cytoplasm. The third zone was characterized by phosphatase-positive, vacuolated cells surrounded by positive matrix. The pericapsular areas contained a higher concentration of enzyme than the remaining matrix. Positive vascular elements invaded the more distal portion of the vesicular zone. From this line of erosion, fairly numerous bony trabeculae extended into the shaft of the ramus with each trabecula surrounded by positive osteoblasts and bone marrow cells. All of the osteocytes showed the presence of enzyme in their nuclei and cytoplasm.

The phosphatase localization in the condyles of the older animals remained typical although certain tissue changes were evident. At 46 days of age, the amount of matrix between the vesicular cells had increased and the trabeculae were coarser. The number of blood vessels impinging upon the cartilage and the size of the marrow spaces had decreased. By 60 days of age, the trabeculae had thickened to such an extent that only a few blood vessels were found at the cartilage edge and the bone marrow spaces were much smaller. The total enzyme reaction of the cartilage and the subcartilage region was less than that of the controls at 25 days of age (Figs. 5 and 6).

Altered Body Growth.—The removal of the pituitary gland brings about a sharp deceleration in growth. In the hypophysectomized rat, formation of

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Plate I

Photomicrographs of sections showing the distribution of alkaline phosphatase in the rat incisor.

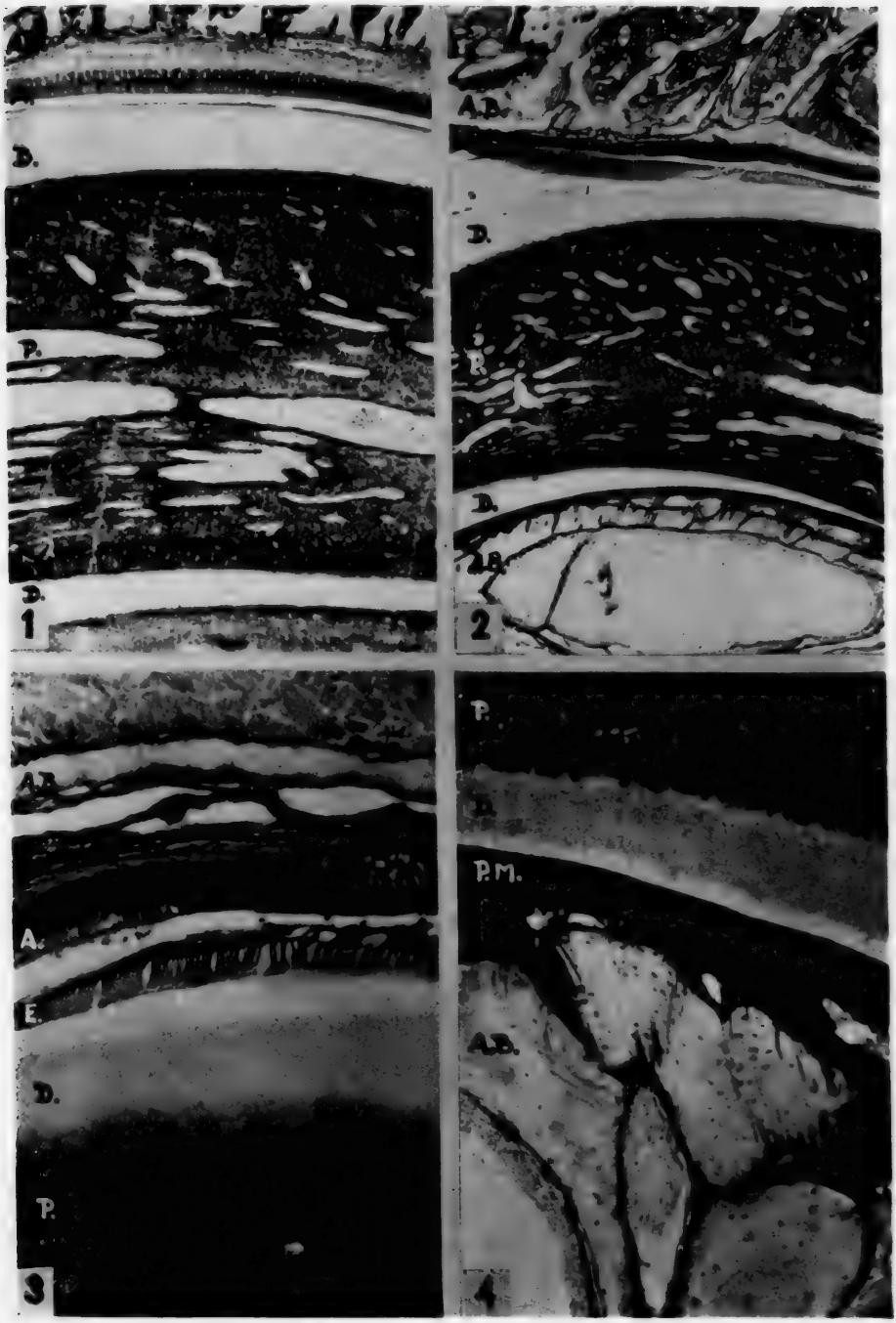
Fig. 1.—Alkaline phosphatase in longitudinal section of upper incisor of 26-day-old control rat. Note wide periodontal membranes. Orig. mag. ×22

Fig. 2.—Comparable area in incisor of animal fed rachitogenic diet for 26 days. The periodontal membranes are narrower than those of controls. The predentin and the osteoid borders of bone show slight graying, indicative of presence of phosphatase. Orig. mag. ×20.

Fig. 3.—Labial aspect of incisor of an animal on rachitogenic diet for 125 days. In addition to its usual localization, enzyme is found in a wide band at the pulpal border of the dentin. Orig. mag. ×80.

Fig. 4.—Lingual aspect of incisor of animal on rachitogenic diet for 125 days. In dentin immediately adjacent to pulp, there is band in which small phosphatase-positive areas are abundant. Sharpey's fibers, osteocytes and osteoid matrix of alveolar bone contain phosphatase. The cementoid is phosphatase-free. Orig. mag. ×80.

A.B.—aveolar bone; *A.*—ameloblasts; *D.*—dentin; *E.*—enamel matrix; *P.*—pulp; *P.M.*—periodontal membrane.



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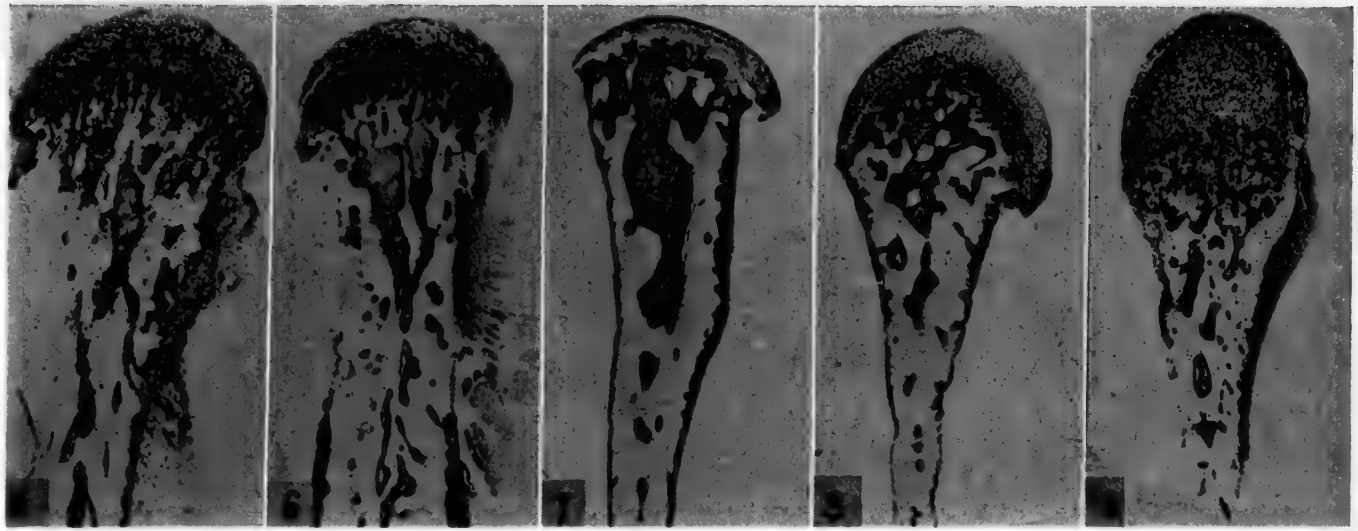

Plate II

Photomicrographs of sections through mandibular condyle showing the distribution of alkaline phosphatase. Orig. mag. ×34.

Fig. 5.—Control rat 46 days of age. There are 3 zones in articulating cartilage, outer zone composed of embryonic or resting cells in which only nuclei display phosphatase, middle zone with both the nuclei and cytoplasm of the vesicular cells containing enzyme, and inner zone with vacuolated cells containing enzyme in their nuclei and with cytoplasm surrounded by positive matrix. Numerous blood vessels with enzyme-positive elements invade distal portion of cartilage. Trabecular formation is regular.

Fig. 6.—Control rat 60 days of age showing narrowing of cartilage and decrease in the number of bony trabeculae.

Fig. 7.—Condyle 14 days after hypophysectomy. Compare Figs. 5 and 6. Cartilage is narrower and regular trabecular formation is lacking. Enzyme-positive elements are greatly reduced.

Fig. 8.—Rat which was given 1 mg. of growth hormone on 15th day following hypophysectomy and sacrificed on sixteenth day. Note increase in cartilage width, renewal of normal erosion and trabecular formation, and concomitant increase in enzyme.

Fig. 9.—Animal fed rachitogenic diet for 35 days. Chronological age of this animal is same as that of control (Fig. 6). Note tremendous hypertrophy of vesicular zone, lack of vascular invasion of cartilage and subcartilage osteoid formation.

Plate III

Photomicrographs of sections showing distribution of alkaline phosphatase in the rat molars and in incisor area of *ia* rat.

Fig. 10.—Mesiodistal section through the molars of control rat 60 days af age. Orig. mag. ×17.

Fig. 11.—Mesiodistal section through molars of animal fed rachitogenic diet for 35 days. Enzyme localization is same as in control (Fig. 10) with exception that there are a few cells containing phosphatase in periapical accumulations of cemetoid tissue. Orig. mag. ×17.

Fig. 12.—Section through incisor area of 12-day-old *ia* rat. At right-hand side is slightly atypical incisor with well-defined morphologic elements. Toward base of this tooth, odontogenic epithelium is folded and displaced. Ectopic formations of dentin and enamel are scattered around the base of tooth. Phosphatase localization is typical in individual tissues whatever their abnormal position. Orig. mag. ×30.

Fig. 13.—Comparable section through incisor area of 20-day-old *ia* rat. Arrangement of tissue elements is just as bizarre as in Fig. 12 but there is increase in quantity of the ectopic formations. Enzyme localization is typical. Orig. mag. ×30.

the bone matrix and tooth substance is slowed down but the mineralization process is undisturbed or disturbed only in the direction of overcalcification. Eruption of the teeth is progressively retarded and ceases at about 8 months following operation.[13]

Hypophysectomy of the short duration used in this study (32 days) had no discernible effect upon the histology of the incisal or molar areas. The distribution and localization of phosphatase were also unchanged.

In the mandibular condyle, on the other hand, the effects of hypophysectomy were quite evident on the fourteenth day following operation. Chondrogenesis was markedly reduced. Narrowing of the vesicular zone and the smaller size of the chondrocytes resulted in a distinct reduction of the width of the cartilage. Regular trabeculae formation was lacking (Fig. 7). At 21 and 32 days, the changes were more pronounced. The phosphatase distribution differed from the normal in that these morphologic changes were correlated with a reduction in the enzyme-positive elements.

Administration of growth hormone to the hypophysectomized animals caused an immediate stimulation of the growth process. The most noticeable response in the jaw bones and teeth occurred in the condyle where the lack of hormone had had the greatest effect. After injection of 1 mg. of the hormone, the cartilage had increased tremendously in width concomitant with an increase in the size of the vesicular cells and the width of the vesicular zone (Fig. 8). In addition, there was a renewal of normal erosion and trabecular formation. The phosphatase content of the vesicular zone increased as the zone widened since each cell contained a typical complement of enzyme. More of the positive vascular elements were present in the subcartilage area. Even with this short period of injection, it was clearly evident that the amount of enzyme present increased only as the phosphatase-positive tissues increased.

Mineral Imbalance.—Definite histological changes appeared in the bones and teeth of animals placed on rachitogenic diet and these became more apparent as the period of rickets was extended. Characteristically constant findings were the increases in the width of the osteoid, dentinoid, and cementoid borders at the expense of the periodontal tissue, bone marrow, and pulp. Calcification and resorption were markedly reduced.

The phosphatase pattern in the rachitic animals differed from the normal due to the fact that the phosphatase-positive tissues were themselves greatly increased or decreased in amount. In the incisors of animals on rachitogenic diet for 26 days, the localization of the enzyme (Fig. 2) was the same as in the control animals (Fig. 1). However, the osteoid border of the bone and the predentin, both of which contained phosphatase, had increased in width and there was narrowing of the periodontal membrane. After 125 days on rachitogenic diet, the matrix of the bone and dentin exhibited a variable but faint reaction throughout (Fig. 3). The dentin was phosphatase-positive to a considerable depth from the odontoblasts, and scattered, highly reactive areas occurred immediately adjacent to the pulp. The cementoid, however, contained no phosphatase (Fig. 4).

In the molar region of the rachitic animals, the enzyme localization corresponded to that found in the controls (Fig. 11). The only additional finding was the phosphatase-positive cells in the periapical accumulations of cementoid tissue.

The histological changes in the mandibular condyle consisted of a tremendous increase in the number and size of the cells in the vesicular zone and the formation of osteoid subjacent to the cartilage (Fig. 9). Phosphatase was present in the nuclei and cytoplasm of all the vesicular cells and in the matrix surrounding them. The osteoid tissue was variably faintly positive. The overall result was a relative increase in the phosphatase reaction of the condyle. These findings were similar to those observed in the reaction of the proximal epiphyseal cartilage of the tibia under the same experimental conditions.[14]

Abnormal Development.—The "ia" rat is a mutant strain with odontome-like formations. The mutation, discovered and bred out by one of us (R. O. G.), is peculiar in the respect that usually only the third molars erupt into the oral cavity. A detailed histological analysis of the dental regions of this toothless rat (Schour, Bhaskar, Greep, and Weinmann[15]) shows that the primary cause of the formations is the resistance of the bone to resorption during the developmental period between the eighteenth day after insemination and the twenty-first postnatal day. Failure of the bone to resorb and accommodate the increasing size of the tooth germs of the incisor and of the first and second molars results in the odontogenic tissues invading the bone marrow spaces. The third molar is less seriously affected because of its later development. Subsequent deposition of dentin, enamel and cementum takes place wherever the formative tissues occur. The end result is a bizarre mixture of all these elements with final ankylosis and lack of eruption (Fig. 12).

From birth through 30 days of age, the localization of alkaline phosphatase was remarkably typical in the individual tissues in spite of the abnormal positions in which the tissue elements were found (Figs. 12 and 13). The reduced enamel organ of the incisor, the stratum intermedium of the developing molars, and the pulp immediately subjacent to the odontoblasts contained the greatest concentration of enzyme. The other tissues were quite comparable to the normal in their enzyme content. It is interesting to note that the osteoclasts which were particularly numerous during the nonresorptive period had enzyme in both nuclei and cytoplasm.

SUMMARY AND CONCLUSIONS

Alkaline phosphatase in the teeth and bones of the albino rat has been investigated in relation to abnormal states of body growth, mineral metabolism, and development. The enzyme localization is unaffected by these diverse conditions but the distribution pattern varies depending upon the morphological changes induced.

Hypophysectomy, by eliminating the source of growth hormone, interferes with growth. Calcification is normal but the rate of apposition is reduced. The phosphatase is noticeably decreased in amount in the synchondrosis of the

condyle where there are fewer vesicular cells. An immediate increase in the number of cells in the vesicular zone follows subcutaneous injection of growth hormone. Concomitantly, the enzyme reaction increases.

Rickets represents an extreme case of abnormal mineral metabolism induced by the lack of vitamin D and a high calcium : phosphorus ratio in the diet. Apposition continues at a normal rate but the newly formed matrix fails to calcify. The total phosphatase reaction under these conditions is relatively greater than normal because of the marked increase in the positive osteoid and dentinoid tissues and in the vesicular cells of the condyle.

In the "ia" rat, abnormal development is caused by a transitory failure in bone resorption. The defect results in overcrowding of the tooth germ with displacement of the odontogenic epithelium and ectopic formation of enamel, dentin and cementum. There is no apparent change in the enzyme content of the individual tissues although their arrangement may be exceedingly bizarre.

From the evidence presented here, it can be concluded that at least some abnormal metabolic states affect the histochemical distribution of phosphatase in the jaw bones and teeth only in so far as they alter the amount of enzyme-positive tissues present.

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SOLUBILITY OF ENAMEL ON AREAS OF KNOWN HARDNESS

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TEETH are often described as being "hard" or "soft." Although this physical property is one often referred to in the literature, it has been difficult to measure accurately. Pickerill[1] was the first to attempt hardness studies on enamel by determining the force necessary to scratch it with a sharp diamond point. Other instruments were tried but because of the extreme brittleness of the enamel, many of the conventional hardness testers were not applicable. Hodge[2] tested the various instruments available at that time and reported the microcharacter to be the one best suited for this purpose. However, it also had definite limitations. The subsequent development of the Tukon tester, employing a Knoop indenter, placed a truly accurate instrument in the hands of investigators.[3, 4]

Since it is well known that enamel is softened when subjected to both organic and inorganic acids, the loss of resistance to an indenting force is one method by which decalcification has been measured. Head[5] devised an instrument for thrusting a plunger, under a given force, into enamel and then measuring the depth of penetration. In this manner he pointed out differences between sound and decalcified enamel, measuring roughly the degree of decalcification. Dobbs[6] and Pickerill[1] scratched the enamel and attempted to measure the decalcification by the amount of enamel removed. Phillips and Swartz[7] later demonstrated, by use of the Tukon tester, a more refined technique which revealed loss of enamel hardness when it was subjected to acid.

However, the most popular method of studying decalcification has been by chemical means. The most accurate for determining solubility is the quantitative analysis for calcium and phosphorus dissolved from the enamel. Most of those studies have made use of powdered enamel.[8, 9, 10, 11] Brudevold[12] used intact enamel surfaces but measured only the amount of phosphorus dissolved. Ericsson,[13] decalcifying much greater areas than Brudevold, determined both the calcium and phosphorus removed from intact enamel.

There have been some attempts made to show what correlation, if any, exists between the physical property of hardness and the chemical property of solubility. Pickerill,[1] whose method has been described, reported there was an inverse relationship between the two. Dobbs[6] indicated that the resistance to acid decreased toward the dentino-enamel junction while Hodge[14] reported the hardness to be less in this area. Klinger[15] however, was unable to show any correlation. He tested the hardness and solubility of teeth taken from carious and caries-immune mouths. Although the average hardness of the caries-immune teeth appeared to be about 25 per cent higher than that of the carious group, no difference in solubility was detected. Karlstrøm[16] showed

*This research supported by a grant (3C4) from the National Institute of Dental Research, National Institutes of Health, United States Public Health Service, Bethesda, Md.

Received for publication, July 20, 1951.

Revised by authors, Jan. 7, 1952.

permanent teeth to be harder than deciduous teeth, and Kanthak[17] utilizing this information, hypothesized that there was no correlation between hardness and solubility of deciduous and permanent teeth.

However, in all of these studies, with the techniques employed, the solubility was not measured on the same areas on which the hardness measurements were made. This is an important variable and could possibly have been a source of error. It is generally known that hardness does vary greatly in different areas of the same tooth[14] as well as from tooth to tooth.[15] The authors have noted in testing hundreds of teeth that even adjacent areas on the same tooth may display great differences in inherent hardness. Thus to correlate accurately the hardness with solubility exactly the same area on the tooth must be employed for both tests. In most of the previous studies described, the teeth were allowed to remain in the decalcifying solutions for extended periods of time. Undoubtedly the acid penetrated to a depth far below the surface where the hardness measurements had been made.

Recognizing these and other variables involved in earlier studies and the lack of a suitable hardness indenter for enamel, it was felt that an investigation should be made to determine accurately whether any correlation exists between enamel solubility and its inherent hardness, employing exactly the same area for both tests. Such data might prove whether a harder tooth, per se, is less soluble.

PROCEDURE

Sound molar teeth placed in tap water immediately after extraction were used in this study. Unfortunately all the hardness testers now available do not permit measurements to be made on a curved surface. For accurate, well-defined indentations, a flat surface must be used. Consequently all tests in this investigation were performed on freshly ground surfaces. Every effort was made to grind each tooth in the same plane so that any difference in hardness due to the direction of the enamel rods would be minimized. This ground surface was prepared parallel to the long axis of the tooth. The technique for sectioning the teeth and the manner in which they were mounted in preparation for the hardness tests have been described in a previous paper.[7]

Since the hardness does vary markedly in different areas of the same tooth, one of the initial problems in this research was to determine exactly how extensive an area of enamel could be employed and still maintain uniform hardness throughout. As large an area as possible was desirable in order that sufficient quantities of calcium and phosphorus could be obtained for the solubility tests. However, as the area is increased there is naturally greater fluctuation in the enamel hardness. An extensive series of tests were devised to determine the maximum area that could be used and yet maintain approximately uniform hardness. After testing hardness throughout concentric circles of varying diameters on large numbers of teeth it was found that hardness values remained quite constant on an area 2 mm. in diameter. This then was the area employed for all tests. It is quite true that some variation in hardness did exist even across this area but the deviation from the average was within acceptable limits.

The decalcifying solution used was .2 molar acetic acid, adjusted to pH 2.5 by the addition of glacial acetic acid. It was necessary to determine the length of immersion time, a minimum time being desired so that decalcification would not progress deeply into areas of enamel having entirely different inherent hardness.

Six teeth were completely covered with wax. A window, 2 mm. in diameter, was cut in the wax on 5 of the teeth, thus exposing the ground enamel surface. One tooth was left entirely covered and served as the control. All teeth were then immersed in test tubes containing the acetic acid decalcifying solution and placed in a constant temperature bath maintained at 37° C. They were removed after varying periods of time ranging from 4 to 48 hours. The control tooth remained in acid for 48 hours. Phosphorus analyses were run on aliquots taken from each of the decalcifying solutions.

The results of these tests showed that 4 hour immersion was not long enough to yield sufficient phosphorus for the analysis while 8 or more hours of immersion produced a measurable amount. Examination of the surface revealed that the acid had penetrated into the enamel quite deeply. In order to decrease the depth of penetration and yet maintain the phosphorus at a workable level, additional teeth with exposed windows were immersed in acid for 6 hours. It was found that a sufficient amount of phosphorus was obtained at the end of this time interval and the corresponding depth of penetration was small. Thus the routine procedure established was to decalcify the 2 mm. diameter area for a period of 6 hours.

The control tooth showed no phosphorus in the acid after 48 hours continuous immersion, and therefore, indicated the wax to be an impervious coating agent for the remainder of the tooth.

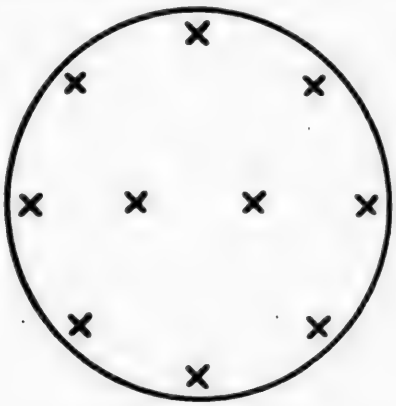

Fig. 1.

In preparing the teeth for the hardness and solubility tests, they were mounted in plaster with the specially prepared ground surface exposed. The entire tooth was then covered with wax and a 2 mm. diameter circle punched in the wax with a leather punch. In this exposed area ten hardness inden-

tations were placed in a pattern as diagramed in Fig. 1. The hardness of a particular tooth was calculated as the average of these measurements. These indentations are microscopic in size and hence it was felt that any change in enamel due to the indenting force would not be sufficient to alter the solubility. After the hardness tests, the exposed window was prepared for the solubility tests by cleaning with a sharpened orangewood stick, cotton, and ether. A binocular microscope was used to make certain that any wax film on this surface was entirely removed and would not interfere with the decalcification. By means of a wire inserted into the pulp chamber the tooth was suspended in the acetic acid for 6 hours at a temperature of 37° C. The solutions were agitated constantly during this time.

Upon removal from the acid the teeth were carefully rinsed with glass distilled water. The decalcifying solutions were filtered by a porcelain filter, diluted to 50 ml., and 5 ml. aliquots analyzed for phosphorus. The method of Fiske and Subbarow[18] was used with a Klett-Summerson photoelectric colorimeter employing a red filter. Calcium was analyzed by the method devised by Solomon, Gabrio, and Smith.[19] It was adapted because of its extreme sensitivity. (The method was modified somewhat as it was not deemed necessary to go through the ashing procedure to oxidize the organic matter. Instead the pH of the filtrate was adjusted and the calcium precipitated as an oxalate by the addition of 2 ml. of saturated ammonium oxalate.)

All water used for making reagents, rinsing, and washing glassware was glass distilled. Care was taken to avoid contamination and to detect any which might have been introduced. Two water blanks were run with each series of tests and analyzed for both calcium and phosphorus. Control teeth completely covered with wax were frequently placed in the acetic acid and aliquots analyzed. At no time was any leakage of the wax coating detected.

Calcium determinations were run in triplicate and phosphorus in duplicate.

RESULTS

Hardness determinations and phosphorus analyses were made on the same area of 88 individual teeth. In order to expediate the progress of this study, calcium determinations were run on only 20 of these teeth. It was thought that phosphorus solubility would be indicative of enamel solubility generally. Brudevold[12] measured only phosphorus but did suggest that a more comprehensive investigation might well include calcium. Results obtained on these 20 teeth should be sufficient cross section to afford a reliable index of the calcium solubility trend of the entire group.

Enamel hardness varied greatly from tooth to tooth as did phosphorus solubility. A graph summarizing the data obtained in testing the 88 teeth can be seen in Fig. 2. Teeth of hardness numbers within definite ranges have been plotted against their average phosphorus solubilities. The softest tooth had a hardness number of 155, while the hardest was 229, a difference of over 40 per cent. From previous experience of the authors in testing large numbers of teeth, the hardness values in this group are felt to be representative. It is recognized

that there are teeth or even areas on these same teeth which vary considerably in hardness but the hardness of the teeth used here is of sufficient latitude to represent a typical cross section.

Fig. 2 illustrates the complete lack of correlation between hardness and solubility. The phosphorus solubility varied widely from tooth to tooth. There was no apparent correlation between the phosphorus dissolved and the inherent hardness. Teeth of almost identical hardness values yielded vastly different quantities of phosphorus. For example, two teeth, with Knoop numbers of 178 and 179, yielded .260 mg. and .720 mg. of phosphorus respectively.

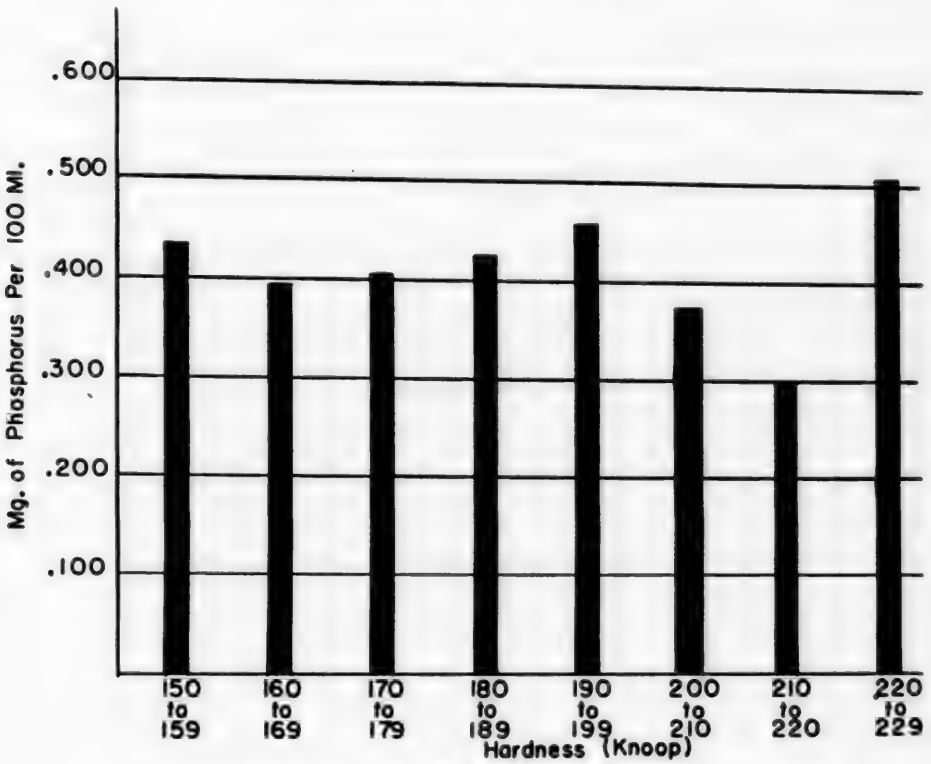

Fig. 2.

The phosphorus and calcium determinations for the 20 teeth which were analyzed for both can be seen in the third and fourth columns of Table I and Ca/P ratios are calculated in the fifth column. There was no correlation between the calcium dissolved and enamel hardness. The average Ca/P ratio for these 20 teeth was 2.38.

As a further check on these data, calcium and phosphorus determinations were run on three different areas of the same tooth. Each area, of course, had an inherently different hardness value. Three teeth were tested in this manner

TABLE I

SOLUBILITY AND HARDNESS ON THE SAME AREA OF 88 DIFFERENT TEETH

TOOTH NO.	AVERAGE KNOOP HARDNESS	MG. OF P PER 100 ML.	MG. OF CA PER 100 ML.	CA/P RATIO
4	161	.696	1.471	2.11
19	176	.716	1.868	2.61
26	178	.508	1.012	1.99
31	179	.380	0.981	2.58
37	181	.848	2.036	2.40
45	187	.445	1.088	2.45
48	189	1.088	2.538	2.33
52	191	.783	1.569	2.00
56	194	.594	1.468	2.47
60	197	.523	1.303	2.49
63	197	.789	1.697	2.15
68	202	.406	1.065	2.62
70	203	.526	1.069	2.03
72	205	.376	0.763	2.03
75	208	.463	1.032	2.23
78	208	.569	1.478	2.59
84	217	.719	1.887	2.62
85	220	.431	1.213	2.82
87	225	.621	1.527	2.46
88	229	.709	1.865	2.63

and the results can be seen in Table II. Again no correlation was found between hardness and solubility. It is apparent from these data, however, that individual teeth do vary as much in the solubility of different areas as they do in hardness. The average Ca/P ratio from these teeth was 2.33.

TABLE II

SOLUBILITY AND HARDNESS ON DIFFERENT AREAS OF THE SAME TOOTH

TOOTH	SURFACE	AVERAGE HARDNESS	MG. OF P PER 100 ML.	MG. OF CA PER 100 ML.	CA/P RATIO
1	A	192	.559	1.540	2.75
	B	186	.162	0.306	1.89
	C	169	1.615	3.243	2.01
2	A	176	.243	.773	3.18
	B	186	.376	.778	2.07
	C	213	.518	1.126	2.17
3	A	197	.293	.741	2.53
	B	188	.168	.376	2.24
	C	211	.400	.843	2.11

DISCUSSION

That teeth vary in their resistance to decalcification has been clearly demonstrated. This supports Brudevold's report[12] on phosphorus solubility. He found that surface solubility varied markedly from area to area on the same tooth. The same phenomenon is apparently true of subsurface of freshly ground enamel. Microscopical examination of these decalcified areas gave further evidence of differences in solubility. Although the solutions were constantly agitated, certain minute areas on some teeth appeared to be almost untouched by the acid. When the depth of penetration was measured the floor of the decalcified area was uneven, indicating that the acid had penetrated to greater depths in some areas than in others.

The Ca/P ratios obtained in this study were somewhat higher than the 2:1 ratio Armstrong and Brekhus[20] showed to exist in enamel. As intimated by Brudevold[12] it appears that calcium and phosphorus are not necessarily leached out of intact enamel at an equal rate. Benedict and Kanthak,[21] using powdered enamel, noted the Ca/P ratio was somewhat high during initial decalcification but later became 2:1 as time progressed. It is true that in the data here represented, some ratios on individual teeth closely approach 2:1 but others were considerably higher. If the solution time was increased beyond the 6 hour interval used here, the ratio might approach 2:1 as shown by Benedict and Kanthak. These results are not in agreement with Eriesson[13] who reported no significant deviation from the accepted ratio in solubility studies on intact enamel. However, he did not give individual determinations but merely stated that, "from untreated tooth surfaces there was dissolved on the average of about 0.12 mg. calcium and about 0.06 mg. phosphorus."

It is, of course, recognized that a harder surface, which is less soluble in acids, can be produced by artificial means, i.e., fluorides.[7] However, from this study, the natural inherent hardness of a tooth seems to bear no relation to its vulnerability to acid attack.

SUMMARY

The hardness and solubility on identical areas of ground surfaces of enamel on a large number of teeth were measured.

The enamel varied widely in hardness and solubility from tooth to tooth as well as from area to area on the same tooth. No correlation between hardness and solubility was detected.

The Ca/P ratio of the enamel dissolved out during the solubility tests was slightly higher than the 2:1 known to exist in ena nel.

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Errata

On page 142 of the February issue of the JOURNAL, under Summary and Conclusions in the article "The Copper and Cobalt Levels of Human Saliva and Dental Caries Activity" by Samuel Dreizen, Henry H. Spies, Jr., and Tom D. Spies, the values for copper and cobalt in saliva should be expressed in micrograms (μg.) rather than in milligrams (mg.).

In the paper by Doctor Robert R. Omata "In Vitro Sensitivity of Fusobacteria to Antibiotics," *J. D. Res.* **30**: 779, 1951, the units of terramycin, chloramphenicol, aureomycin, tyrothyricin, dihydrostreptomycin, and streptomycin are given in Table I (page 800) as mg. for ml. These units of measurement should read "micrograms per ml." instead of milligrams (mg.).

In the paper by Duncan McConnell, "The Crystal Chemistry of Carbonate Apatites and Their Relationship to the Composition of Calcified Tissues," *J. D. Res.* **31**: 53, 1952, the group symbol in the bottom line of page 56 should read "$C6_3/m$."